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PROCEEDINGS OF THE CONFERENCE ON PHYSIOLOGICAL ASPECTS OF WATER QUALITY HELD IN WASHINGTON, D. C., 8-9 SEPTEMBER 1960

Johns Hopkins University
Baltimore, Maryland

1960

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PROCEEDINGS

Conference on Physiological Aspects of Water Quality

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September 8-9, 1960

Washington, D.C.

240

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PROCEEDINGS

Conference on Physiological Aspects of Water Quality

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this document may be better
studied on microfiche

September 8-9, 1960
Washington, D.C.

PURPOSE OF THE CONFERENCE

This Conference on Physiological Aspects of Water Quality was organized to stimulate research interest in the physiological aspects of certain chemical constituents of water. The program was planned to focus attention on questions of what is known and what should be learned about the effects of minerals, trace elements, insecticides, and organic substances in potable water.

It was recognized that almost no information is presently available concerning the physiological effects of consuming, over long periods of time, water that contains either minute amounts of potentially toxic chemicals or excessive amounts of the common minerals. The effects may be beneficial at certain levels and harmful at others, as is the case with fluorides.

Many of the chemical constituents of water, both natural and man-made, have never been identified. In the case of organic substances, even the techniques for identification remain to be developed. The detection and analysis of micro-chemical concentrations, however, has been made possible by recent developments in instrumentation.

A small "working conference" of scientists selected to represent a variety of disciplines was planned. The program was designed to provide reports on the status of research information and to suggest the directions of future investigations. Obviously, a larger number and a wider variety of future studies must be undertaken to explore problems of this nature. The maximum value of the conference on physiological aspects of water quality, therefore, will result from wide distribution to additional scientists of the information presented in the form of published Proceedings.

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ACKNOWLEDGEMENT

Support for the Conference on Physiological Aspects of Water Quality and for publication of the Proceedings was provided by Research Grant RG-7748 awarded by the Public Health Service to Dr. John C. Geyer, Chairman, Dept. of Sanitary Engineering & Water Resources, The Johns Hopkins University.

MESSAGES

From the Public Health Service

"I have a great interest in the subject of this conference, and hope that it will be productive of much increased activity in the study of what I am convinced is a most important problem."

Dr. David E. Price
Deputy Director
National Institutes of Health

"There seems little question that scientists should focus their attention on the physiological aspects of new contaminants in water. It is apparent that the classical toxicological approaches to problems of this nature will not provide the intelligence we need to solve them. I am confident that these conference deliberations can stimulate new research efforts leading to a better understanding of the microchemical constituents in our drinking water."

M. D. Hollis
Assistant Surgeon General
Chief Engineer

"We recognize the need for more basic knowledge in all areas of water supply, and especially in the protection of water quality. Research underway in this field is small in relation to growing problems. I anticipate that this conference will indicate the directions in which we must expand our studies of pollutants in public water supplies."

Gordon E. McCallum, Chief
Division of Water Supply
and Pollution Control

VI

Conference on
Physiological Aspects of Water Quality

PROCEEDINGS

Edited by

HARRY A. FABER

and

LENA J. BRYSON

contents

CONCERNING THE CONFERENCE - Purpose of the Conference iii

Acknowledgement iv

Messages v

OPENING STATEMENT - by Gerard A. Rohlich 1

KEYNOTE ADDRESS - by Robert A. Kehoe

Human Health and Well-Being and the Aqueous Environment 3

PAPERS ON WATER QUALITY PROBLEMS

The Need For Water Quality Criteria - by J. E. McKee 15

Detection and Analysis of Chemicals in Water
I. Inorganic Constituents - by M. G. Mellon 27

Detection and Analysis of Chemicals in Water
II. Organic Constituents - by F. M. Middleton 39

PAPERS ON MINERALS AND TRACE ELEMENTS

Occurrence of Trace Elements in Water - by W. H. Durum 51

Quantitative Mineral Requirements - by D. M. Hegsted 67

Effects of Trace Amounts of Molybdenum - by Kenneth Monty ... 75

Effects of Trace Amounts of Selenium - by Klaus Schwarz 79

Effects of Trace Amounts of Vanadium - by H. E. Stokinger ... 105

Effects of Trace Amounts of Zinc - by W. E. C. Wacker 113

Open Discussion 115

PAPERS ON INSECTICIDES AND ORGANICS

Physiological Effects of Insecticides - by W. J. Hayes 129

Physiological Effects of Organic Phosphate Pesticides - by O. G. Fitzhugh 135

Physiological Effects of Insecticides on Aquatic Life - by C. H. Hoffmann 145

Open Discussion 161

PAPERS ON ORGANICS

Minimal Effects of Organic Water Contaminants - by J. A. Zapp 167

Chronic Toxicity of Dodecylbenzene Sodium Sulfonate - by O. E. Paynter 175

Cancer Hazards From Natural and Artificial Water Pollutants - by W. C. Hueper 181

Open Discussion 195

REVIEW SESSION - SUMMARY STATEMENTS

Minerals and Trace Elements - by J. L. Whittenberger 205

Insecticides - by Arden R. Gaufin 209

Organics - by Norton Nelson 215

National and International Standards of Water Analysis - by F. W. Gilcreas 219

Report on Research Project at University of Pittsburgh - by M. A. Shapiro, et al ... 223

CONFERENCE EVALUATION - by Abel Wolman 233

DIRECTORY OF PARTICIPANTS 239

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OPENING STATEMENT

By

G. A. Rohlich

The basic concept of a conference planned to delineate the physiological and toxicological effects of chemical constituents of water was proposed by Dr. Jack E. McKee, Professor of Sanitary Engineering, California Institute of Technology, past chairman of the Environmental Sciences and Engineering Study Section, National Institutes of Health, Public Health Service.

A planning committee of Study Section members was appointed to develop the program for this conference. It included the following members:

Dr. John C. Geyer, Chairman
Mr. Harry G. Hanson
Dr. Robert A. Kehoe
Dr. T. E. Larson
Dr. C. N. Sawyer
Dr. J. L. Whittenberger

The entire membership of the Study Section assisted in the selection of speakers for the conference. Staff assistance for conference arrangements was provided by Mr. Irving Gerring, National Institutes of Health, and Mr. Harry A. Faber, Division of Water Supply and Pollution Control.

The specific aim of the conference was summarized in the following statement:

> "To investigate the present state of knowledge with respect to the physiological effects of certain chemical constituents of water, to discover if possible what types of research can and should be undertaken in order to determine both the adverse and beneficial effects of such chemical constituents, and to learn how they may be altered or eliminated in the interest of creating a healthier environment."

Plans for the conference were discussed with representatives of the Office of the Surgeon General, National Institutes of Health, and Bureau of State Services of the Public Health Service. They recognized the broad environmental health implications of the information to be derived from a meeting of this type, and provided valuable guidance to the planning committee.

There has been a lack of interest, until recently, in the non-biological substances in potable water. While chemists and engineers have given some attention to the micro-chemical constituents there is an evident need for wider physiological and toxicological evaluation of these problems. The speakers selected for this conference, therefore, include physiologists, toxicologists, and biologists in addition to chemists and sanitary engineers.

In opening the conference, I wish to express appreciation to the members of the planning committee of the Study Section, and to the Public Health Service members who provided staff assistance. I wish to recognize the contributions of speakers who have prepared the technical papers comprising our program. Finally, I wish to welcome all the invited participants and to request that they offer their questions and comments as a part of our conference on the physiological aspects of water quality.

KEYNOTE ADDRESS

HUMAN HEALTH AND WELL-BEING AND THE AQUEOUS ENVIRONMENT

By

Robert A. Kehoe, M.D.

A prior conference on "Man Versus Environment," sponsored by the National Institutes of Health on the recommendation of the Study Section on Sanitary Engineering and Occupational Health, was held in May of 1956, to consider certain potentially hazardous features of the modern human environment. In its consideration of these features, which included the pollution of the air, the processing of human food, and the contamination of the water supply of mankind, it dealt largely with the role of modern technology in creating threatening conditions in the environment, and the need for extensive and sustained research in bringing these conditions under rigid scrutiny so as to provide the information required for their recognition, appraisal, and control.

The Composition of Water is Important

This conference on the "Physiological Aspects of Water Quality," conceived of and sponsored in a corresponding manner, has taken but one environmental medium as its theme, in an effort to focus the attention of experts on one of the principal hygienic problems of all time, including the present - namely, the quality of the water which should be made available to man in accordance with his physiological needs. At first blush, this seems a sharp limitation of the subject matter as compared to the earlier conference. Further consideration, however, serves to show that this is not true or, at least, that the limitation is more apparent than real. For there is no specification that can be applied to water for human consumption, with respect to physiological criteria for its chemical composition, that dares to ignore the contribution made to the regular human intake of these components through the food and the atmosphere. Clearly, the moment one includes the food in this consideration, he must recognize that the composition of the water which will best maintain life in the flora and fauna of the earth, is the subject of discussion. This is in keeping with the evidence of the origin of life in the waters of the planet, and of the persistence of a medium very like that of the sea as the stream along which, and by reason of which, the cells of the complex organisms on land and sea are nourished.

Four Questions to be Considered

There are four questions, at least, to which attention must be given. What are the desirable components of water for human consumption, as prescribed, but not always provided, by nature? What is the optimum range of concentration of each of these components, whether supplied wholly, or now only in part, by nature, in water for drinking and culinary purposes? What changes in the composition of the human water supply have been affected by human artifice? To what extent are these artificial changes harmful?

There are, of course, many other questions, collateral to, or derived from, these questions, and as indicated previously, there is no answer which applies satisfactorily to this environmental medium in the absence of corresponding information on other contributory media in the human environment. If, however, our inquiry be limited essentially to these four questions, we shall have the substance for a discussion that will take us backward into the earliest periods of biological history, and forward into the future of man's efforts to create an environment subject to his control and compatible with his physiological well-being.

Our four questions will also seem to have excluded any reference to the quantities of water which may be required now and in the future. Even this, however, is not strictly true, for it will be seen that the quantities of water that are available generally and locally have much to do with the concentration of the various constituents derived from washing the atmosphere and bathing the land.

Every factor that alters the precipitation of water upon the earth and into the sea; that changes the amount and duration of its retention in the soil and in the underground pools, and, conversely, that influences the rate of run-off or escape into the streams that flow into the sea; every such factor affects, likewise, the components of man's water supply and the concentration of the components therein, whether such supply derives from reservoirs of water within or on the surface of the land, or from the natural or artificial streams that traverse it. If we are to do anything about the quality of our water supply, we must know not only its composition, and what its composition should be, but also the factors in both nature and artifice which can be counted on to influence its quantity as well as its composition.

Desirable Physiological Properties

At this point we should examine our knowledge as to the desirable "physiological" properties of water for human consumption, and the sources of such knowledge. Certainly much must be known about the purity of water. With all that has been believed, spoken and written about health-giving springs and spas; with the extensive commerce in bottled waters, some of which are merely regarded as clean and palatable, while others containing natural salts and, expecially, radioactive components, are said to cure disease and restore health; acknowledging the virtues of clear, cold water from a mountain stream, a sylvan spring, the cool well with its "old oaken bucket," the clean cistern with its pure supply of rain water, and, latterly, the water from the treatment plants that supply the urban dweller with pure water from the faucet; surely with all of this lore, all of this wisdom of daily experience, and all of this science of water treatment, we must know what is meant by "purity." Alas, we do not, except in a negative sense.

The water we drink should not contain pathogenic viruses or microorganisms, parasites, or the ova of such parasites. This is certainly what we know best, and the most precise and effective tests, specifications and processes of treatment are directed to the fulfillment of this criterion. Here and here only is where science has been brought to bear effectively upon our practice. For most of our guidance, otherwise, we depend upon our senses, individually and traditionally. Drinking water, for example, must not taste bad or, at least, too bad.

The presence of excessive quantities of iron salts, which is fairly readily recognizable, may offend our sensibilities in this regard, but since we have been told that no harm is likely to come from its presence, such water can be drunk, and even relished if sufficiently cooled. Drinking water must appear to be clean. Suspended material causing mudiness or milkiness, in greater or lesser degree, is repellant, although it will not prevent the drinking of water in extremity. The color of water is also important, quite apart from its taste. The brownish-black waters of the swampy areas, with their relatively high content of ground acids and tannins, are unattractive even when one believes them to be harmless. Water must not be excessively salty.

Salinity up to a point, which varies considerably with the state of the body and the ambient temperature, is acceptable and even, at times, highly refreshing. While there is little question that here is a physiological appetite, mediated, it seems, by the same type of primitive stimulus that draws herbivora to salt licks, it is, so far as man is concerned, a sensibility which lacks much in discrimination. Except for the striking bitterness and astringency of certain salts - notably certain salts of magnesium, iron and aluminum - the only factor which exercises a decisive effect upon the palatability of saline waters seems to be the concentration of the saline components, individually, or collectively. There may, perhaps, be an additional or complimentary factor in saline water, or at least a factor to which reference must be made, in that naturally or artificially carbonated waters have long had a strong appeal to certain palates.

Whatever the truth may be in this latter respect, the minerals in drinking water have an ancient and persistent appeal, not only to the taste of man, but to a sense of well-being which is derived, mainly, one may suspect, from a strong faith in its health-giving properties. This, like the belief in God, seems to have been a nearly universal faith among men. How much of this stems from a worshipful attitude toward nature, how much of it is a product of witchcraft and the healing arts of primitive man, would be hard to say. It has had support in evolutionary doctrine on the one hand, as well as in the classical experiments that have given us physiological salt solutions of somewhat variable but similar composition, in which isolated living cells from complex organisms survive for considerable periods, whereas they die promptly in distilled water.

The Concept of Purity

The preceding point return us to the purity of water, which, in the technical sense, is a complete misnomer, for if chemical purity were to be specified, a carefully controlled type of distillation would appear to be the method of choice in the preparation of drinking water, and efforts would be made to reduce to a minimum the inclusion of all other substances in the course of its distribution and use. What is meant in common parlance by the term, "purity," is either an aesthetic quality - the appearance of cleanliness - or a high degree of freedom from organic material and the associated micro-organisms of filth and sewage. In much of the past history of mankind, this concept of the purity of drinking water has been fairly satisfactory.

In the era of scientific hygiene, however, for the purposes of the optimum in human nutrition, as well as for the avoidance of the natural occurrence in water of various components and products of the earth and its living forms, in objectionable or harmful concentrations, the specifications for water as a beverage must be more meaningful and more exact. What is the role, for example, of the minerals? One fairly dramatic example of where we are in this respect and of where we are likely to be in the future in controlling the concentration of mineral elements, is offered by the present controversy on fluoridation. Those who will have it that God created the waters of the earth, that, they are good, and that man, with his limited and finite knowledge, alters them for general use recklessly and in disregard of personal freedom and public peril, will continue to ignore all evidence to the contrary, and hold that we should in this respect prefer nature, despite her manifest disinterest in our ultimate fate, to our own admittedly experimental, but disciplined efforts toward health and survival.

I. The Experimental Approach to Qualitative Specifications

The first stage in the exploration of the qualitative characteristics of water for human use and consumption would appear logically to be in the examination of the vast store of the recorded observations concerning the waters of the earth; their varied and varying composition; their movement in the atmosphere; their condensation and precipitation; their infiltration into, through, and within the soil and subsoil; their collection in surface, subterranean and oceanic reservoirs; their surface run-off; their leaching through the soil; their progressive drainage into streams of increasing volume; their eventual running down to the oceans with their residual burden of leached out minerals and their suspended solids, all the while, in certain areas, and at times in others, being immobilized in varying quantities as snow and ice, and all the while, also in varying degree, being returned to the atmosphere as water vapor.

Natural Variability of Composition

No one can review, even in a superficial manner, the crude facts concerning the complex pattern of the circulation of water around, over and through this planet - the hydrologic cycle - without recognizing that the quantities and qualities of water available for human use in all parts of the earth, save only the oceans and certain other very large and comparatively remote bodies of water, are subject to tremendous variability from time to time and from place to place. Gross variations are induced by natural causes. An excess of evaporation over precipitation and the inflow from streams, concentrates the constituents of water in the surface and underground reservoirs, and if this condition persists, prolonged aridity, with salt lakes or salt deposits, results.

An excess of precipitation, on the other hand, in the worst areas, may result in the denudation of the earth through the runoff into streams, bearing with them scores of tons of soil per square mile per year. Between these extremes of aridity and wetness, a degree of variation may occur at various places on the globe and for periods of time varying from the immediacy of a drought or flood to the longer or shorter cycles discerned in the world's somewhat obscure climatic history. In this pattern the composition of the water in every trickle, brook, creek, or river, and in every puddle, pond, lake or inland sea, as well as in the corresponding superficial and deep underground reservoirs, differs from every other, dependent upon the composition of the soil and ground over which or through which the water has passed.

The variation is the greater, generally, in the inverse relation of the volume of water to that of the land mass, and the only reason that oceans are so little affected is that their volume and their burden of dissolved materials is so great (the latter being the accumulation of millions of years) that the 3,000 million tons of salt poured into them annually is too little to affect a significant change in concentration except in sharply localized areas and for short periods of time.

It is little cause for wonder, considering the varied composition of the earth's soil and crust, of its underground deposits in certain areas, as well as the concentrating effect of solar evaporation on the dissolved substances in land-locked bodies of water, that some of these natural waters are unfit for consumption by man and animals, and that, in some of them, aquatic life is sparse or practically non-existent. Indeed the number of possibilities for variation in chemical composition, with respect to the mineral components alone and their respective concentrations, is almost infinite, being limited only by the essential insolubility of many of the more toxic minerals.

Artificial Variability of Composition

Still further variations in the composition of water have been and are being introduced by human activities. Some of the latter have had remarkably little effect on water because of their confinement to superficial layers of the earth. Thus it is apparent that mere tillage of the soil, throughout the greater part of historical time, while having a fairly profound effect upon the land, has exerted little influence on the quality of the water, locally or generally. On the other hand, the modern use of fertilizers, pesticides and insecticides has affected a highly significant degree of contamination of surface waters in the areas concerned, in especial relation to the decimation of aquatic life.

More profound and more lasting effects have been brought about by certain human activities which alter appreciably the hydrologic cycle in localized or widespread areas. Indeed it can be said that every use of water by man, even for such purposes as navigation, the production of hydroelectric power, or recreation, reduce the quantity available for other purposes and impair its quality in some degree. Such uses in the United States as those represented in irrigation and in public supplies have more obvious and more diffuse effects, involving, as they do, some four to six percent of the volume of our flowing streams.

Effect of Storage on Water Quality

The storage of waters in various ways, and the regulation of the flow of streams, impound large volumes of water, which, themselves, undergo little change - much less than that which would occur during the normal cycle - but these procedures increase, to the degree of their scope and volume, the changes which affect the quality of that which is not impounded. The extensive development of wells, and the use of ground waters, lower the water table, divert water from streams, alter its vaporization, and, where practiced near a sea coast in great excess of replenishment by precipitation, may so exhaust the supply as to bring in a tide, so to speak, of salt water, with perhaps a permanent change in the quality of the ground water of the area.

The disruption of the drainage of an area, as by the diversion of the flow of a stream for irrigation, may bring about a striking change in the underground storage of water in the original area, and, perhaps, a permanent loss of storage capacity, as well as a profound change in the quality of the water left in the original channel. This may also alter drastically the quality of the diverted water after it has traversed its new drainage area.

The extensive conversion of forest land to the purposes of agriculture increases the run-off and the drainage, reduces the water-holding capacity of the ground, increases the suspended metals in the streams, and results in a fall of the water table. In addition to a decrease in the volume of the water available locally, changes in its quality are induced by its different pathway over and through the ground into the streams, and by its shorter stay in the ground.

Coverage of the ground with the impervious structures of towns, cities, other buildings, and the vast stretches of intervening highways, removes land increasingly from the leaching effects of rainfall, while adding foreign material increasingly to the waters which drain back quickly to the streams.

The Discharge of Waterborne Wastes

All of these, and many more, are the acts of man, and the effects of his increasingly active occupancy of the earth in growing numbers. And last, but certainly not the least of these, is his use of water and waterways - most of the streams, and certainly the preponderance of their combined flow - for the disposal of the general wastes of community life and much of the liquid and solid wastes of modern industry. Among the latter, the problems of the disposal of the radio-active wastes of general and specific industrial processes involving nuclear energy are upon us, not in their full magnitude but in most of their potentialities. They point up, as nothing else could, the gap that lies between man's immediate urge for action and his full knowledge of the consequences of such action, in the disposal of wastes, generally.

Hitherto, in the activities of peace, as in those of war, men have believed that they could forge ahead, follow their main purpose, and disregard the collateral consequences until such time as the latter might reach proportions requiring some diversion of effort to meet and retard them, or perhaps to deal with them satisfactorily. Such a course, in the somewhat clearer light of retrospection or of history, has been seen at times to be a monstrous error, characterized much less by a commendable boldness than by a collossal foolhardiness. Such wisdom as our generation of men can muster for its guidance has warned us that this attitude cannot be allowed to prevail in dealing with types of energy which, if loosed among us, may destroy life in wide areas of the earth, or alter it in imponderable ways.

Such is the power of the mysterious threat of radioactivity that this advice is heeded, and most men are willing to be told what to do to be saved. This is good, provided enough of the truth can be learned soon enough for practical application to our peace-time activities. And herein lies the clue to our action on a much broader base than that of acquiring a comprehension of the biological effects of atomic radiation.

Pandora's Box of Chemical Pollutants

Scientific man has released not only atomic energy from its natural confines, in concentrating it in his service, but he has also opened up a virtual Pandora's box of chemical agents with the idea of employing them for his convenience, comfort and enrichment. Only in the immediate sense, has he perceived their potentialities for harm, and only recently has he developed some awareness of the consequence of their distribution upon the earth, into its waters and into its atmosphere. As yet, however, he has not gone far enough in his recognition and limitation of their spread.

On the other hand, it is likely that these contributions to the hazards of human life, significant as they seem in experience and anticipation, are not as much of a threat to life on this planet, and perhaps to that of man, as are the disruptions in the balance of nature induced by the man-made changes in the face of the earth, in the disposition of its land and its waters to serve human purposes. Many such disruptions that have been brought about may have to be corrected at great cost, and certainly, in the future they should be anticipated and accepted or counteracted.

It has been my privilege to sit in the councils of the Study Section that conceived of this conference and the one which preceeded it. Here in fields foreign to my experience, I learned something of the complexities of aquatic life, something of the interrelated participants and events in the biology of streams, something about sewage, mostly organic sewage, and something about the reclamation and use of waste waters.

This was a rewarding but an humbling experience, for I saw again, as I had seen before in exchanges between men of wide knowledge and vision, that the search for knowledge, while eminently successful, had but opened up still larger fields for further inquiry. But to learn something more of the dynamic processes of life, and to be aware of the greater expanse of the unknown, is not necessarily to be discouraged or deterred, but perhaps, more often, to be challenged. This has been the role of man since first he ate of the tree of knowledge and acquired an undying taste for it.

II. THE EXPERIMENTAL APPROACH TO QUALITATIVE SPECIFICATIONS

The second step toward the achievement of realistic and satisfactory specifications for the quality of water is that of renewing our experimental search for more, better, and more pertinent information concerning the waters of the earth, the environmental factors that affect their composition, and the influence of the components, in the concentrations in which they occur singly and collectively, upon the health and well-being of man.

Here there is an abundance of work to be done by an army of investigators in different fields of inquiry - work in such quantity as to strain our combined resources, and of such a range of quality as to satisfy the most pedestrian as well as the most agile of minds. Indeed we cannot afford at this juncture to take lightly the contributions that can be made by honest investigators with modest competence and slight experience.

On the other hand, the urgency of the situation is such that we cannot afford to leave more than we must to chance in our experimental strategy. There is need, therefore, to examine the many problems in the light of the combined knowledge and skill of the many scientific disciplines that have been involved, at any point, thus far in their investigation. Herein is the function of this conference.

A Coordinated Study of Problems

The problems to be investigated need to be presented clearly so as to challenge systematic and effective inquiry. They need to be examined for their impact upon, and their relation to, various scientific disciplines, not for the purpose of our "riding off in all directions," but rather in order to achieve such a degree of coordination that each of us can work within his capabilities, but not in ignorance of the capabilities and skills of others. Something of the relative importance of the various problems with which we are confronted should emerge from these discussions.

Perhaps, also, some of those who have reason to perceive the immediacy of some of our perils, may also recognize voids in our basic knowledge of underlying geologic, geographic, hydrologic or biologic phenomena, which are far beyond their own capacities to explore, but may well be within the competence and methodology of others in our present scientific constellation.

ADDENDUM

Here, then, is the task which has been laid before us in the form of a program to be followed. My function has been the modest one of expressing the general purpose of this gathering, of posing some questions to those who have been called upon for advice and help, and of inviting the up-surge of interest and enthusiasm which may promote present and future inquiry.

By way of conclusion, I should like to draw attention to a recent article by Colin Kytle in the Saturday Review of August 27, 1960, and point out its kindred relationship to our present scientific situation. Kytle's concern is with the moral and spiritural consequences of the Technological Revolution in America, which, whatever else it has accomplished, has brought to many persons a sense of frustration in that "the only certainty is that things will change a great deal faster tomorrow than they are changing today."

Kytle quotes Margaret Mead as saying that "We've grown oppressed by a sense that the world(has) gotton so big and unmanageable that it(is) very hard for individuals to influence it very much." The term which applies to this feeling, according to Kytle, is the sociologist's word, alienation, which may be defined as "powerlessness, to the sense of being controlled by alien forces, of being unable to determine the outcome of events. (It) also refers to meaninglessness, to the sense of being involved in complex, unintelligible affairs. And, finally, alienation refers to what we might call self-estrangement, to the sense of being unable to realize a directly satisfying self-image, of being other-directed, of being involved in work in which intrinsic meaning has been lost in routine."

The Control of Events

The cure for this aimless, unrewarding prospect, according to Kytle, may well come from some distinctively inspiring popular movement that will restore to the American people the sense that "events can be controlled, even when their effects cannot be foreseen." Without appearing to prescribe a better or more definite remedy, one might suggest that one of the first requirements toward a greater maturity in the large segment of our population that is so clever and yet so bewildered and undirected, is that of developing a sense of personal responsibility.

In shifting the emphasis from this general theme to our more limited concern, let us recognize that it is not a disembodied technology that is affecting the on-rush of changes in the physical, social, and intellectual climate of the present world. Rather, it is the individual minds of men, fascinated by their own cleverness, and heedless of the consequences of the pursuit of their own purposes wherever they may lead, that have brought us to this pass.

Responsibilities of Technical Minds

It is time, high time, that technical minds should become adult minds, should become responsible and should seek by the same or similar methods that have created change, to discern the remote effects of such changes in the environment and in the life of man, present and future, and to plan and carry out appropriate action accordingly. For example, to the extent that the productive and distributive activities of industry damage the soil, poison the water, and pollute the atmosphere, industrial technologists are responsible for knowing that these things may occur, and for anticipating and avoiding them.

Industry is made up of people who are responsible for their own acts; these include some of the best of our scientifically trained persons, who are responsible in their own personal and professional right, but who, in addition, can secure such advice and guidance as may be required to act with prudence. It should be recognized that such responsibility is being accepted by industry. One of the most refreshing signs in our present national life is the extent of this acceptance.

There is, indeed, a growing recognition among technical and scientific people, generally, in industry, in schools and universities, and in governmental circles, that we can determine the immediate and remote consequences of our technical activities, to a very large extent. We need not, and dare not, trust to time and luck. Our's is the responsibility for seeking and knowing the truth about this physical world in which we live, lest our destruction should result from our ignorance of natural law, on the one hand, and from the work of our own hands in distorting or thwarting her law, on the other.

It is here, one may hope, that a stronghold of confidence in the future of mankind and in his capacity for self-discipline and self-direction, may be erected. Even one such stronghold, well sustained, might prove to be a rallying point for a renewal of the courage and faith of our confused and anxious people.

THE NEED FOR WATER QUALITY CRITERIA

by

J. E. McKee

Let us examine the title of this paper, "The Need For Criteria of Water Quality." Who has the need and why? How can the need be defined? How urgent is it? Why hasn't it been filled before? Why do we say criteria? Why not speak of the need for data, or standards, or objectives?

It is germane for this conference to examine these questions and especially to determine if there really is a need for criteria of water quality. It is also important to describe some of the problems facing waterworks authorities and regulatory agencies in the management of this Nation's water resources.

This paper is intended to explore some of these problems and to present some background information for the control of water quality. The subject is a broad one, with many ramifications; hence, the paper serves primarily to introduce the problems with no attempt at exhaustive treatment. Subsequent speakers will provide many of the details.

The Need for Criteria

Who needs criteria of water quality and why? Criteria are needed, first, by water pollution control authorities who must restrict or regulate the discharge of sewage and industrial wastes to the Nation's water courses and groundwater basins. Second, criteria are needed by waterworks engineers and chemists who are responsible to the consumer for the quality of treated and delivered water, despite any pollution of the source.

Similar parameters are needed by aquatic and marine biologists who strive to protect fisheries resources and by irrigation engineers who are responsible to the farmer for the quality of water delivered to crops. Finally, criteria of water quality are needed by public-health authorities and by engineers in environmental health who are concerned with all of man's contact with his environment.

Conventional Criteria Available

What criteria are needed? What analyses are important for the protection of our water contact? These are questions that this conference might well attempt to answer. At present, chemists and microbiologists in water supply organizations and regulatory agencies are conducting the analyses that they know how to perform or, more specifically, they are running the tests that are conventional. It is possible, indeed probable, several important trace constituents are being overlooked, and conversely some of the conventional tests are relatively meaningless for the protection of public health.

To get a thorough picture of water qulity, however, we need a comprehensive compendium of criteria to assess the effects of each potential pollutant on each possible beneficial use of water.

Such a tome was prepared at Caltech for the California State Water Pollution Control Board in 1952, with an addendum in 1954. The demand for this volume was so heavy that a second printing was ordered in 1957. It has gained worldwide acceptance, including translation and publication in Japanese. The second printing is exhausted, but rather than reprint again, the California WPC Board in collaboration with the U.S. Public Health Service preferred to revise and modernize the text. A project is now underway at Caltech to prepare a second edition which should be ready in 1962. Considerably larger than the first edition, for data are accumulating rapidly, this volume will be a collation and evaluation of all readily available criteria for many potential pollutants as they relate to each beneficial use.

More Comprehensive Criteria

In spite of the rapid accumulation of data relating to water quality, the collection and collation of available information will reveal that there are many gaps in our knowledge of this vast subject. Among the missing information, the following examples are prominent:

1. Effects of synergism and antagonism among potential pollutants.
2. Rates of decomposition of unstable substances under various conditions.

3. The relationships of criteria of water quality to the phenomena of diffusion, dispersion, and transport of wastes, such as longitudinal and transverse mixing in streams.

4. Threshold concentrations for the thousands of exotic new organic chemicals that are being conceived by manugacturing chemists, used by industry or agriculture, and allowed to reach water courses.

5. Radioactivity from waste waters and from fallout, as it affects beneficial uses.

6. Subtle long-range effects, expecially on biological systems and specifically on human physiology.

7. Limitations of available water quality data, based on inadequacies of sampling and questionable analytical procedures.

8. Daily, hourly, and even instantaneous variations in the concentrations of specific substances, which might be revealed by instruments for continuous sampling and recording.

9. The feasibility of using mathematical models to describe water quality parameters, coupled with computer technology and systems analysis.

Urgency of the Need for Criteria

That the need exists for better criteria of water quality is beyond contention, but how urgent is this need? Until recently, many people believed our water resources, except in the arid West, were abundant and virtually inexhaustible. The flow of these waters has been measured for many decades by the U. S. Geological Survey, and the probable flow in future years can be predicted. One thing is certain. Barring a major change in climate and geology, the folume of our water resources will not increase. Indeed, it may even decrease slightly as more reservoirs permit higher evaporative losses and as more water is used consumptively. Attempts are being made to increase the supply of fresh water by desalinization of sea water, by cloud seeding, or by suppression of evaporation. It is unlikely, however, that these measures will provide a sizable increase in the total fresh water resources.

In contrast with a fixed supply of water, we are faced with increased demands from a growing population, expanding industry, and complex changes in technology. Concomitant with the expanding demands for fresh water of good quality are the increased discharges of waste products to the nation's water courses. Worse still are the magnitude and variety of new substances being discharged - substances about which little is known in relation to their effects on beneficial uses. To recapitulate, the supply of water remains constant, the demand for it increases, and the quality deteriorates. Can there be any question that the need is urgent for additional information relative to the effects of water quality on beneficial uses?

Analytical Techniques and Instruments

Why hasn't this need been filled before? One reason has been the lack of an acute urgency. More plausible, however, is the fact that, until recently, analytical techniques and instruments for measuring many of the pollutants were not available. Where once the water chemist relied on simple physical and chemical tests, he now has at his disposal equipment such as the flame photometer and the infra-red spectrometer to measure trace elements, molecular-filter-membrane techniques for bacterial and biological analyses, adsorptive devices such as the carbon filter for microanalysis of oderiferous compounds, liquid-and gas-chromatographic procedures, and several other recent improvements.

While many of these techniques are still confined to research laboratories, their impact will soon be felt in waterworks practice. Most important, perhaps, is the trend toward electronic instrumentation to provide continuous analyses and graphic records of water quality. Here the waterworks engineer has much to learn from equipment manufacturers and from other branches of engineering and science.

Significance of Trace Substances

It is significant to note that sanitary engineers and chemists were the first to worry about the presence of trace minerals and exotic organic substances in domestic water supplies, and they were first to recognize the inadequacies of present knowledge about the effects of these substances on human physiology and other biological systems. Perhpas the fears of the engineers and chemists are unfounded; but until this area of ignorance is resolved, they are justified in calling upon physiologists, biologists, and medical doctors for assistance. That is one of the reasons for this conference.

It is natural for waterworks engineers and chemists to be concerned about trace substances in their product, especially the new and exotic ones. They are also intrigued, however, about the possibility that some constituents of water, whether natural and conventional, or foreign and new, may actually be beneficial to the consumer. Experience with fluoride for humans and borates for crops has suggested the possibility that the discharge of certain wastes to our water courses may be beneficial, rather than deleterious, to downstream users. Certainly more information is needed in this area.

Criteria and Objectives vs. Standards

The title of this paper and the foregoing discussion deal with criteria. Why not speak of standards or objectives?

The term "standard" applies to any definite rule, principle, or measure established by authority. The fact that it has been established by authority makes a standard somewhat rigid, official, or quasi-legal; but this fact does not necessarily mean that the standard is fair, equitable, or based on sound scientific knowledge, for it may have been established somewhat arbitrarily on the basis of inadequate technical data tempered by a cautious factor of safety. Where health is involved and where scientific data are sparse, such arbitrary standards may be justified.

A prime example of a standard that was set somewhat arbitrarily is the limit of 0.05 mg/l of hexavalent chromium in the U.S. Public Health Service Drinking Water Standards of 1946. The previous limit, in the 1942 standards, had been 0.00; but one of the members of the committee on revisions operated a water system in which some of the wells had 0.02 or 0.03 mg/l of hexavalent chromium. To get his wells back in service, he prevailed upon the rest of the committee to raise the standard to 0.05 mg/l--just high enough for his wells to qualify. Little or no scientific information was available at that time to suggest a proper threshold value, but in the interests of safety the committee wanted to keep the standard as low as possible.

Another example, based on ignorance coupled with caution, is the arbitrary limit for coliforms in saline bathing waters, as promulgated and rigidly enforced by many state health departments. Epidemiological evidence is much too sparse--indeed virtually non-existent--to justify these standards on a scientific basis; yet they provide a strong administrative crutch to bureaus of sanitary engineering that want to promote a high degree of sewage treatment, regardless of economic justification.

The word "objective" represents an aim or a goal toward which to strive and it may designate an ideal condition. Most certainly, however, it does not imply strict adherence nor rigid enforcement by an agency or health department. It is gaining favor among engineers on boards and commissions that strive to achieve water pollution control by persuasive methods and cooperative action. An example would be the objectives for various reaches of the Ohio River, as promulgated by the Ohio River Valley Water Sanitation Commission.

The Value of Criteria

A "criterion" designates a means by which anything is tried in forming a correct judgment respecting it. Unlike a standard it carries no connotation of authority other than that of fairness and equity; nor does it imply an ideal condition. When scientific data are being accumulated to serve as yardsticks of water quality, without regard for legal authority, the term "criterion" is most applicable.

To be useful, a criterion should be capable of quantitative evaluation by acceptable analytical procedures. Without numerical criteria, vague descriptive qualitative terms are subject to legal interpretation or administrative decisions. A criterion should also be capable of definitive resolution, i.e., unaffected insofar as possible by synergism, antagonism, and other complicating factors. There is a tendency, which should be avoided assiduously, to let criteria become rigid and perhaps ripen into standards. For this reason, every criterion should be regarded as flexible information, to be kept constantly under surveillance.

Potential and Corollary Pollutants

Definitions are also in order for the terms "potential pollutant" and "corollary pollutant." Any substance that may be present in water, whether dissolved, colloidal, or suspended, is a "potential" pollutant--potential in the sense that if concentrated sufficiently it can adversely and unreasonably affect such waters for one or more beneficial uses; and yet, if diluted adequately, a "potential pollutant" will be harmless or perhaps even beneficial. A good example is the fluoride ion.

The term "corollary pollutant" relates to a substance or living organism of natural origin that is not normally deleterious, obnoxious, or adverse to the beneficial uses of the water, but may become so when altered or stimulated by the introduction of sewage or industrial wastes. The discharge of ammonium phosphate, for example, may not in itself cause any direct pollution or toxic action; but it may stimulate heavy algal blooms with concomitant fish kills. In a similar fashion, Sphaerotilis may become obnoxious as a result of concentrations of sugars that are not in themselves deleterious.

Man's Impact on His Sources of Water

The invluence of civilization on a natural water course may be exerted in various ways and in many degrees. First to warrant consideration, perhaps, is the development of natural resources. Deforestation of the virgin trees and reforestation with a new species may alter the quality of surface drainage and ground water by changing the forest ground cover, by decreasing transpiration, and by increasing evaporation. The uncontrolled development of mineral and petroleum resources may result in gross pollution of surface streams and underground basins, as it occurs in hydraulic mining, by acid mine drainage, and by the discharge of oil field brines. Even the harnessing of water power by the impoundment of surface streams will have an effect on the quality of the water.

Agriculture represents a second major impact on water quality. Even in the absence of irrigation, the cultivation of land affects the quality of surface and subsurface waters. When irrigation is practiced, these effects generally are intensified. Natural return flow or artificial drainage from irrigated fields will contain dissolved minerals in excess of those in the applied water, the type and magnitude of such increases being a function of the crops grown, irrigation practices, climate, soil characteristics, and similar factors.

A major factor in modern agriculture is the use of pesticides and herbicides. These compounds are generally some form of halogenated hydrocarbons and they are remarkably stable. Dusted or sprayed indiscriminately on fields, some of them may reach water courses directly or they may be washed in by a subsequent rainstorm. In natural waters, they may be directly toxic to fish or to the insects on which fish feed. Worse still, they may reach domestic water supplies, where their effects are not well known.

Effect of Wastewater Treatment

The growth of agriculture and the development of natural resources lead to concentrations of population and the water-carriage system of waste disposal, the third major impact. Fortunately, the practice of discharging raw sewage, without treatment, is rapidly diminishing. It must be recognized, however, that no practical degree of treatment will restore used domestic water to its original quality. Even with complete biological stabilization, the total dissolved solids may be expected to increase by 100 to 300 mg/l, corresponding to about 70 lb per capita per year. Despite this increase, there are instances where treated waste waters may be superior in quality to alternate sources of supply. For this reason, reclaimed waste waters should always be considered as one of the potential sources of fresh water in arid regions.

A minor factor in the quality of raw water sources, but one that cannot be ignored, is the demand for and the effect of recreational use of surface waters. When fresh-water streams and lakes are limited, they cannot forever be denied to the public for fishing, boating, and even swimming. In the past, many cities have relied upon protection of watersheds to preclude the need for treatment in excess of chlorination. Such protection, at best, is risky and difficult to enforce. Cities must face the changing concept of watershed protection, share the limited facilities for recreational purposes, and be prepared to extend the degree of water treatment. This does not mean, of course, that open distribution reservoirs or other impoundments close to the consumer should be exposed to contamination by recreational use. Somewhere between the concept of completely protected watersheds and that of contaminated distribution reservoirs rests the equitable solution to this problem.

Variability of Industrial Wastes

Finally, liquid wastes from certain industrial processes may exert a tremendous effect upon water resources. The number, variety, and character of such wastes are too complex to describe here. Of significance, however, is the fact that these wastes are highly variable from industry to industry, and from period to period at the same plant. The development of new processes and the synthesis of novel compounds require that water pollution control authorities be constantly alert to the hazards of new waste waters.

Fluoride complexes, hydrazine compounds, acrylic acid derivatives, complex pertochemicals, and radioactive materials are but a few of the substances whose disposal constitutes industrial-waste problems.

The net effect of man's multifaceted impact has been, and will continue to be, a deterioration of the quality of natural fresh-water resources, whether surface or underground. This is a concept that all must recognize and face. The trend is inevitable as long as population continues to increase and industralization is intensified. The trend must be combated and slowed by rigid control of industrial wastes, by regulation of resources development, and by treatment of municipal wastes, but it cannot be stopped or reversed and its ultimate development cannot be prevented unless the industrial and agricultural development of this Nation is to be curtailed. Allowance must be made in our water works planning for this eventual impairment.

Not All Pollutants are Deleterious

Owing to this inevitable deterioration of water quality, it is more important than ever to know which pollutants are deleterious, and which are relatively harmless or perhaps even beneficial. In this manner, the major effort of pollution abatement can be directed at the most serious problems. As a corollary, some control measures might be relaxed if it can be shown that certain waste substances are not harmful and may even improve the quality of water for certain beneficial uses. For example, the discharge of nitrates and phosphates might increase the fish productivity in a stream without harming other beneficial uses.

Trends in Quality of Domestic Water Supplies

Man's original criteria of water quality were simple indeed; they consisted largely of physical tests such as temperature, taste, odor, color, and turbidity. Although old and familiar to the layman, these criteria scarcely deserve the faith placed in them by a Mississippi jurist in 1904 when he ruled, in part, "It is not necessary to weigh with tenderness and care the testimony of experts. Any ordinary mortal knows whether water is fit to drink and use."

Chemical criteria of water quality were proposed almost two centuries ago. Early observers, for example, viewed organic matter in water with suspicion. They attempted to determine it quantitatively and relate it to the incidence of disease. That human and animal wastes could contaminate water and render it dangerous has been known for centuries, but not until after van Leeuwenhoek developed the microscopy of water were bacterial and biological yardsticks formulated.

Criteria and analyses of water quality have progressed remarkably in the past century and even in the past two decades. Perhaps the best and most recent evidence of our requirements for water quality is the Drinking Water Standards of the U. S. Public Health Service. The fact that these standards and the methods of analysis are revised and improved every 5 to 10 years is ample proof of our progress in water quality criteria. Each change is attributable to advanced knowledge of the effects of various substances on the many beneficial uses of water.

Not all changes are in the direction of stricter standards. Some of the earlier requirements were unduly restrictive because the thresholds of toxicity or other detrimental effects were unknown. The interim standards, as a result, reflected a conservative approach. As research unfolded the true effects, these conservative standards were relaxed. In 1942, for example, the U.S. Public Health Service Drinking Water Standards restricted copper to 0.3 mg/l. By 1946 this requirement had been relaxed to 3 mg/l.

"Luxury" Standards of Quality

Our concepts of the quality requirements for finished waters appear to be shaped by several trends. First, as the standard of living rises and the "luxury" market expands, there is a continuing pressure for water of the highest possible quality. For this reason, imperfections in treated water delivered to consumers are seldom tolerated, especially by the housewife. Silt in the laundry tub or automatic washing machine, iron or manganese stains on the porcelain of the wash basin, discolorations in a pitcher of water, or a ring around the bathtub are frowned upon by modern Americans who expect, and rightfully demand, the highest possible quality.

This trend in the luxury market is illustrated by the increasing popularity of household water softeners, despite a frequently heard contention that the new synthetic detergents (syndets) make softening unnecessary. Syndets are used extensively for dishwashing and home laundries, but they have not replaced soap in the bathtub, shower, or washbasin, where the hardness of water becomes most evident to the user. Soft water is still a luxury much desired by housewives for washing hair, skin, and nylon fabrics. Indeed, many commercial laundries and restaurants still favor soft water and soap over synthetic detergents for washing clothes and dishes.

"Additive" Standards of Quality

The second trend that may alter our concepts of finished water is the demand for additivies to improve the quality of water for a specific purpose. Illustrative of this trend is the campaign by dental and public health authorities for fluoridation of water to supplement our fluoride-deficient diets. The municipal water supply has been designated by American public health agencies as the chosen vehicle to deliver approximately 1 mg of fluoride per day to each child.

Fluoride and, formerly, iodide are but the first of many trace elements that might be added to water. It is not difficult to envision traces of cobalt or copper that might improve bone structure or skin. Perhaps 0.5 mg/l of boron could be added to make grass grow better on watered lawns. Indeed, the day may not be distant when odor-masking or deodorant compounds are added to improve the taste of water. Certainly, a slight peppermint taste would be preferable to that of dilute hydrocarbons. Truly, the role of trace substances represents a new frontier of American life, and the water supply may be chosen as the vehicle to deliver some of these substances to every household.

"Industrial" Standards of Quality

Another trend that will shape thoughts about the quality of finished water is the increasing proportion of municipal water supplies being used by industry. Several cities can be cited where the population is declining but total water use is skyrocketing as a result of the demands by industry. Waterworks engineers must give increasing attention to the primary need of industry for water of a quality that remains relatively constant over reasonable periods of time. Most industries are willing to accept municipal water that meets drinking water standards, with the recognition that this water must be altered for specific industrial purposes such as boiler feed, cooling, or process water. Industry is quite prepared to accept and further treat municipal water, provided that such water is not subject to sudden changes in quality.

To epitomize these trends and concepts in the quality of finished water, it is apparent that consumers, both domestic and industrial, will continue to demand improved quality and greatest univormity. To meet these requirements, the waterworks operator must start with sources of raw water that are likely to deteriorate in future years. He must, therefore, produce a better and more uniform product on the one hand while drawing upon a poorer source of supply. Fortunately, he can call upon many recent improvements in technology that are capable of bridging the widening gap in quality between raw and finished waters. His greatest need today is knowledge relative to the potential harmful effects of trace substances in the sources of water.

Summary

This paper has attempted to describe the problems facing sanitary engineers and chemists in the control of stream pollution and the maintenance of optimum quality in domestic water supplies. It has pointed out several areas of ignorance and it has emphasized the concern of waterworks authorities over the possible deleterious effects of exotic new substances in the Nation's water resources. Perhaps this conference will help to focus attention on these problems and lead to research that will provide many of the solutions.

DETECTION AND ANALYSIS OF CHEMICALS IN WATER

I. INORGANIC CONSTITUENTS

By

M. G. Mellon

The determination of various constituents in water has long been a problem of analytical chemists. Thus, two of the best known colorimetric methods, those for ammonia and iron, were proposed more than a century ago.

Currently the analysis of water is a very important problem. A rapidly increasing population and an expanding chemical industry must be adding to our water supplies contaminants in ever greater amount and variety. Even to look at many of our creeks and small rivers today gives one a bad taste sensation. The production of potable water from some of these supplies is an outstanding chemical achievement one which has been almost unmentioned in present chemistry teaching and is appreciated by few people using the water.

Analytical Methods Available

Two aspects of this problem are analytically important. First, most of the contaminants usually occur in relatively small amounts. This often requires special techniques for their detection and determination. In a few cases there seems to be need for a method capable of determining an amount approaching 1 part per billion. Second, there is a great diversity of soluble inorganic chemicals which may reach many of our water supplies. Included are at least elements, ions, radicals, compounds, and isotopes, some of which may be radioactive disintegration products. Presumably sea water contains the greatest variety of dissolved substances. Often contributing to the difficulties with these inorganic constituents are various organic contaminants which may interfere with at least some determinations.

The detection and/or determination of all possible inorganic contaminants in water, in a wide range of relative concentrations, is a formidable analytical problem. Many articles and books, especially in the last quarter of a century, have dealt, either directly or indirectly, with water analysis as such, or with methods of possible or probable interest. A valuable series of papers was published as a book, "Trace Analysis" (8). Of special value are the annual reviews in Analytical Chemistry.

Considine's book (1), "Process Instruments and Controls Handbook," is of particular interest for equipment and apparatus which may be used in monitoring and/or controlling the composition of process streams. Included is at least the semi-automation of the determination of substances in a series of samples on a routine basis. An accompanying chart summarizes, for 104 instruments and technics, a large amount of valuable information on general application limits, physical technics employed, and practical instrumental considerations, such as availability, sensitivity, response speed, sampling requirements, and installation and maintenance. It may be noted that there are not 104 different kinds of methods. Many of the entries are really subdivisions.

Problems in Quantitative Analysis

Currently questions such as the following seem to be relevant. What is the present problem? What is in water? What needs to be determined? What technics are available? What are their limitations? Are any promising developments likely?

This is a large order. The author presumes only to present a personal, brief perspective of the over-all problem, with attention centered on quantitative analysis. Emphasis should first be placed on the indispensability of knowing what is in a sample before applying quantitative methods. Since most such methods are non-specific, it is very hazardous to apply them without knowing that the system is free of possible interfering constituents.

The author admits uneasiness here. Which of our methods are reliable in the presence of any possible combinations of contaminants? Do we need to do a preliminary qualitative analysis, or at least determine that possible interferences may be neglected? Do we have adequate precautions of this nature in our approved methods? If we do not need a comprehensive qualitative scheme for water, do we not need at least recommended tests for possible interferers in connection with the quantitative method for each constituent? Also what about the purity of reagents and possible contamination of the reacting systems during an analysis?

It is not easy to select the items appropriate for this short presentation. More or less arbitrarily the speaker has attempted to direct attention to the nature of the problem, to the processes most used to render a constituent measurable, and to the more common operations and technics applicable for the measurement.

Preliminary Treatment of the Sample

Depending upon the nature of the sample, one or more preliminary treatments may be necessary prior to separation and/or measurement of a desired constituent. The most common processes are adjustment of pH, change of oxidation state, and complexation. Samples such as sewage require prior preparative treatment.

Complexation concerns us most here. The process is used to render an interfering constituent non-interfering or to make a desired constituent more readily separable and/or measurable. For example, in the absorptimetric determination of manganese in the presence of much iron the highly colored Fe^{+++} ion may be converted to the colorless FeF_6^{---} ion, and the nearly colorless Mn^{++} ion to the highly colored Mn_4^- ion.

Developments in this area have been extensive in the past decade. Included are the proposal of new complexants, application of them to many situations, determination of stability constants, and study of the nature of the structures of complexes.

Separation of Desired Constituent

Whenever possible, a desired constituent is determined in the sample as submitted. Often, however, this is not possible because: (a) one or more other constituents interfere with the measurement, or (b) the concentration of the desired constituent is too low to be measured by the given method. For example, the interferer may absorb radiant energy at the same wavelength as the desired constituent, and the 1,10-phenanthroline-iron complex is not sufficiently sensitive for the absorptimetric determination of 1 part per billion of iron in water in a 1-cm. cell.

It may be noted that separations are always objectionable because they require time, equipment, and skill. Often, too, they are the most likely sources of error. However, they seem inevitable until we devise interference-free methods of measurement.

A brief statement follows on the processes of separation most commonly utilized.

Volatilization. Volatilization is a very old process of separation. Well known applications in inorganic analysis are boron as methyl borate, fluorine as silicon tetrafluoride, and nitrogen as ammonia.

Leslie and Kuehner (2) have a recent review of advances in apparatus and applications. Striking examples of the latter are various isotopes and the isolation and recovery of amounts of material of the order of 10^{-9} gram.

Volatilization of a major component to make a minor one measurable is of some importance. Thus, a water sample may be evaporated considerably to bring iron up to a readily determinable range. This process will not work, however, for residual chlorine ammonia, carbon dioxide, and dissolved oxygen because of loss of the desired constituents. Oxidizable constituents, such as ferrous and nitrite ions, probably would be at least partially oxidized.

Precipitation. This is another very old separative process. For small amounts of desired constituents the chief items of interest are the continued search for precipitants yielding a more nearly complete separation, a more nearly pure precipitate, or a more effective coprecipitation. The last process, often referred to as "gathering," is probably adsorptive in nature. Sulfate precipitated as barium sulfate in the presence of ferric iron is likely to be contaminated with the iron.

Few analysts like to precipitate, filter, wash, heat to constant weight, and weigh, and they would welcome elimination of precipitation.

Electrodeposition. Although nearly a century old, separation by electrodeposition has had little application to water analysis. It is readily applicable only to a few elements, such as copper, cadmium, cobalt, and lead. Even these are not easily handled in minute amounts without special equipment and care.

Liquid-liquid extraction. Recent years have brought many advances in methods involving the differential solubility of substances in liquids. Chiefly this is the result of the present availability of many organic solvents and the increasing rise of complexants to produce systems readily extractable (4).

In water analysis this process is common practice for some of the metals, such as cadmium, copper, iron, lead, nickel, and zinc, and for the non-metals arsenic, phosphorus, and silicon when handled as heteropoly blues. In some cases the complexant is included in the extractant.

Probably still other solvents will prove of value, and possibly the technic may be made more efficient, although the counter-current modification is already an elegant process.

Adsorption. The condensation of gases, or of substances in solution, upon solids in contact with them is known as adsorption. Familiar examples are the adsorption of certain gases in masks and of the Fe(III) ion by precipitating barium sulfate.

There are various modifications in adsorptive technics. The adsorption of ferric ions on barium sulfate exemplified concentration of a minor constituent. In differential adsorption a solution containing two or more solutes may percolate down through a column packed with adsorbent, or the solution may diffuse through adsorbent paper, with or without an impressed direct electrical current. After separation, the components are measured by appropriate means either in situ or after removal by eluents.

Chromatography, although a very poor term, is a technique widely used in connection with these and other processes, such as ion exchange. In the latter technique, an ion of the solution exchanges for one from the adsorbent.

In so-called gas chromatography, the mechanism of separation seems not to be entirely clear. It seems to be partly extractive and perhaps partly adsorptive in nature. At present the technic is very popular. It is restricted to substances readily volatilized and to those that are stable when volatilized at evelated temperatures.

In his recent review on chromatography, H. H. Strain (6) stated "....this technique, in its numerous modifications, is by far the most widely applicable separatory method." No doubt it could be applied to waters for at least part, if not all, of the components. Considerable time and effort would no doubt be involved, at least when many constituents are desired.

Measurement of Substance

Measurement is the final and inevitable manipulative operation in determining the amount of a substance in a sample. Some property of a system is measured. The system may be the desired constituent itself, although more often it is something having a known chemical relationship to the desired constituent.

More than a score of methods of measurement are now available, and many of them are applicable to water analysis, at least under certain conditions and for part of the constituents. Which of these are preferable depends upon circumstances. Small laboratories will not have adequate emission spectrometers or micro equipment for gravimetry and titrimetry. Very few have access to a source for neutron activation methods. A completely equipped modern analytical laboratory is expensive to install, to operate, and to maintain. Brief comments follow on a number of methods.

Gravimetry. Since all substances in water possess mass, they may be determined gravimetrically, provided (a) they can be isolated and maintained in measurable form and (b) a sufficiently sensitive balance is available. Difficulties with the first step are so great with some substances that this method is not used for them. Ultramicro balances are applicable to very small masses.

Gravimetry is still recommended in "Standard Methods for the Examination of Water and Wastewater" for various constituents. However, because of the separative difficulties involved, this technic seems likely to be less and less used as others can replace it.

Volumimetry. The volume of gases, liquids, or solids can be measured. However, this possibility has been little used in water analysis because of obvious difficulties of isolating the constituents in measurable form.

Manometry. Manometry is limited to measurement of substances in the gaseous state. No applications seem to have been made to practical water analysis, although the technic is well known in biochemistry.

Titrimetry. For at least a century titration has been a widely used method of measurement. Its application in water analysis is well known. On a mass basis the measurement can have high reliability.

The main requirements are suitable titrants, interference-free reactions, and means of detecting end points. Significant developments have occurred in the use of certain complexers as titrants (such as ethylene-diaminetetraacetate), and in instrumental detectors for end points.

These instrumental end point detectors include at least the following types: potentiometric, conductometric, photometric, thermometric, and currentometric. In all of these it should be noted that calculation of desired constituent still involves only the amount of titrant used.

Unless such means increase the precision of a measurement or contribute to possible automation of the titration they have little merit where visual detection is satisfactory. Instrumentation leads sooner or later to maintenance problems or other instrumental troubles.

Spectrometry. Spectrometric methods are based on the measurement of various kinds of spectra. The most used spectra are the result of selective absorption or emission of radiant energy by many substances. The past two decades have brought vast improvements in spectrometers and their greater availability in many laboratories.

Various applications of these have been made to water analysis. The types of spectrometers noted are generally most important.

1. Emission Spectrometers. Every analyst knows the flame test for sodium. The two emission lines at 5890 and 5890 Å is a very sensitive test for this element, and the intensity of the lines indicate its amount.

Excitation in some kind of flame and measurement with a photometer, such as the Beckman B, is often referred to as a flame photometry. Usually only a few elements, such as the alkali and alkaline earth metals, are easily determined with this relatively simple and inexpensive emission equipment. Use of high temperature flames promise more extended applications.

Much more elegant and expensive instruments, using high temperature arc or spark excitation enable one to determine probably all of the metals, and under special conditions, part of the non-metals. The highly automated direct readers are invaluable for routine analysis of many samples of the same type. These instruments, often called spectrographs, are expensive and limited to elements. Their value, for both qualitative and quantitative analyses, would seem to justify more extensive application whenever they are available.

Late developments in these types include rare gas atmosphere in the arc chamber and special electrode designs. Various results in parts per billion are reported in metal analysis.

Atomic absorption spectroscopy, a closely related technic, is based upon the fact that metal atoms absorb at the wavelengths of the emission spectral lines of the particular metal. This new technic has been compared with emission technics and its advantages noted (2,5).

2. Absorption Spectrometers. Molecular absorption spectrometry has extensive applications for the determination of inorganic substances. Absorption spectra are widely used for qualitative identification, and the Bouguer-Beer law relates the magnitude of absorption of radiant energy, at particular wavelengths, to the amount of the substance absorbing.

Very largely the applications are to trace amounts, but differential measurement has been used in the visible range for elements in major amounts.

Measurement of absorption spectra is usually considered in terms of the region of the spectrum involved. Partly, at least, this has resulted from variation in the sources, optics, and detectors used.

In polycomponent systems, such as water samples, interferences are widespread. When two or more substances absorb at the wavelength to be used, the desired constituent must be separated or the interference taken into account in some manner.

a. Infrared region. For various reasons infrared spectrometry has not achieved wide use for analyzing water, although it is a very sensitive method. Possibly dissolved gases, such as ammonia, carbon dioxide, chlorine, and oxygen could be volatilized and so determined, but the separative problems are not easy.

b. Visible region. Measurement in the visual region is widely practiced for some two dozen substances. Included are anions, cations, and dissolved gases. Few of these absorb sufficiently as such, and there are various interferences. However, various kinds of separations and/or complexations have provided reasonably simple procedures.

The sensitivity for several substances is approaching 1 part per billion. The situation is still improving with the development of more sensitive instruments and the discovery of more highly absorbing complexes.

c. Ultraviolet region. Excellent instruments are available for the non-vacuum ultraviolet region, but applications in this region are much fewer than in the visible region. Some complexes absorb best here, and probably in some cases interference in the visible range can be avoided. Perhaps further study will reveal other possibilities.

d. Other regions. Measurement in other regions may be of interest, at least in some situations. Thus, gamma-ray and x-ray spectrometry, which employ very short wavelengths, seem to be increasingly important.

Electrometry. Electrodeposition is a separatory process. Electrometry is a kind of measurement involving some electrical property of a system.

1. Potentiometry. Although appealing in principle, application of the Nernst equation for measuring the concentration of various substances by means of electrode potentials has been disappointing. The activity coefficient is not known in practical situations.

Some determinations have been done by empirically calibrating with known mixtures. The common meter for measuring pH value is the chief practical example. Even here we must be content with an operational definition. We do determine a very useful quantity, but it is a meter reading on an instrument checked against known buffer solutions.

2. Polarography. In this type of method one measures current for given potentials impressed on the electrode pair used. This method has been widely studied and there are many oxidation-reduction applications.

As the generally usable range of concentrations is around 10^{-5}M, many constituents in water in the range of a few parts per million or less are not determinable. If the constituents, such as metallic ions, can be concentrated, as by evaporation, they can be so determined.

3. Coulometry. Coulometry, like titrimetry, involves determination of the amount of reactant necessary to complete a given chemical reaction. In this case the actual measurement is coulombs (current x time); that is, the number of electrons required to complete the reaction. Reactions may be deposition on an electrode, dissolution from an electrode, or generation of a substance which reacts with the desired constituent. Assuming that Faraday's law applies, the amount of reactant may be calculated.

Detectability is said to be of the order of 10^{-12} equivalents of reactant. Methods have been proposed for a considerable number of substances, usually in simple systems. It is not clear to the author what may be done with a complex water sample without making at least some separations.

Fluorescimetry. Closely related to absorptimetry in the visual region (colorimetry) is fluorescimetry. Many substances, especially when treated with suitable reagents and irradiated with ultraviolet radiant energy, fluoresce strongly in the visible region. Thus, uranium, a striking example, is detectable in minute amounts.

Much work has already been done (7). Recent instruments, such as the spectrofluorescimeter, will refine and no doubt extend the applications. Water analysis may well be among them.

Radioactimetry. Perhaps the most sensitive of all technics is based upon the detection and measurement of the products of radioactive disintegration of atoms.

Only a few of the elements, such as potassium and uranium, occur naturally with radioactive isotopes. Many others become radioactive as a result of the fission process which occurs in atomic explosions and reactors. Strontium-90 may be the most publicized of these products.

Because of possible pollution of waters with products of atomic "fall-out" or of wastes from reactors, determination of such products may be necessary.

Activation of non-active substances has become a valuable technic for many elements. No doubt its applications will increase as activators become more readily available.

Other Methods. Still other methods of measurement have some application for trace amounts of various inorganic substances. Among these are mass spectrometry, thermal conductivity, electrical conductivity, and certain micro technics. No doubt there are more.

Very interesting, and often important, determinations can be made in these ways. Thus, mass spectrometry and thermal conductivity are used in gas chromatography. Because of the present state of their applications to situations such as the analysis of water, they are not considered here. They may well be of value for this purpose, especially for investigating research problems.

Conclusion

The determination of all inorganic substances which may be in water, in all of the possible relative amounts, is a difficult analytical problem. No one type of method will always be suitable. Depending upon the composition of the water and the requirements for individual determinations (such as cost, time, skill, instruments, sensitivity, and reliability), the most appropriate methods depend upon the properties of the substances to be measured. To the author's knowledge, all present methods have not yet been adequately applied to practical water analysis.

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DETECTION AND ANALYSIS OF CHEMICALS IN WATER

II. ORGANIC CONSTITUENTS

By

F. M. Middleton

The interest in organic contaminants in surface water supplies has classically been focused on domestic sewage or other biological degradable materials related to the dissolved oxygen of the stream. Physiological considerations were confined to bacteria or other organisms and to some metals. Chemical contaminants were not believed to be of physiological importance in the early days of sanitary science. This is no longer true. Even the 1946 Public Health Service Drinking Water Standards, which are currently under revision, mention only phenol and heavy metal gluosides as organics that are to be limited in drinking water.

It is the purpose of this paper to review the time-honored tests of water quality and to point out new approaches pertinent to our assessment of the organic contaminants that are now believed to affect it. Gaps in our knowledge will be mentioned.

Organic Analytical Techniques Up to Now

The biochemical oxygen demand (B.O.D.) test has been in use for over half a century. It measures the quantity of oxygen required to oxidize the decomposable matter subject to aerobic destruction by organisms under the conditions existing during the test. The test, while useful for its intended purpose, does not tell what is present and does not measure inert or highly stabilized organics.

The chemical oxygen demand (C.O.D.) test is a measure of the oxygen required to oxidize those substances that will be attacked by the oxidant, usually a potassium dichromate solution in sulfuric acid. While this test measures some of the organic materials in water, the nature of the materials is not revealed and most aliphatic and aromatic hydrocarbons are not attacked.

Organic nitrogen as measured by the Kjeldahl method has been used in stream work to measure the nitrogen in the form of proteins and intermediate decomposition products. Combined with tests for other forms of nitrogen, information is gained about the nature of pollution.

Phenolic materials came to the attention of water users early in the history of industrial pollution because of their unique properties of increasing in taste and odor intensity upon chlorination, and because of the extreme disagreeableness of the chlorinated products. As a result of flagrant taste and odor episodes and the sizeable sources of phenol pollution, much attention has been given the development of analytical methods for these compounds. In the 1959 Analytical Chemistry Review on Water, 6 of the 13 references relate to phenol. Standard tests will measure a few micrograms of phenolics per liter of water, but the individual phenols are not separated and some varieties are missed.

"Standard Methods for the Examination of Water and Sewage" (10th Edition, 1955) provides a test for tannins and lignin. Based on the reduction of phosphotungstic and phosphomolybdic acids to produce a blue clor, this test measures materials having aromatic hydroxyl groups but does not distinguish among such substances.

Except for tests applied to sewage and wastes for measuring oil and grease, volatile matter by ignition, and volatile acids, "Standard Methods" goes no further in describing organic analytical techniques. Other tests for organics are available and find use in special situations. Demand has not put them into the standard procedures.

Need For New Analytical Techniques

To our knowledge, acute or immediate physiological damage to humans has not occurred as a result of the consumption of organic materials in water purveyed to the public by water utilities. However, information gained from examination of water for organics suspected of causing other water damages leads us to believe that careful assessment is needed of possible long-term physiological effects from organic contaminants. Population growth, industrial expansion and the growing use of chemicals in the home and on the farm support this view. The function of the chemist here is to bring to the attention of toxicologists the presence of materials which may be suspect, and to seek sensitive and definitive procedures to measure contaminants that require evaluation.

Application of new techniques and extension of old methods to the organic contaminants in wastes and waters have revealed in the past ten years that:

1. New types of organic materials -- detergents, insecticides, and miscellaneous petrochemicals -- are appearing in surface and ground waters. The concentrations are low; usually not more than a few milligrams per liter (ppm) and often only a few micrograms per liter (ppb).

2. Concentrations of contaminants while low, are sufficient to cause water damage such as undesirable taste and odor in drinking water, off-tastes in fish, fish kills, fouling of ion exchange materials, foaming, water treatment difficulties, and interference with recreational uses of water.

3. Many contaminants are resistant to the normal processes expected to remove or eliminate them. The best sewage treatment still leaves residues of organics. One synthetic chemical waste, orthonitrochlorobenzene, was detected in the Mississippi River at its point of discharge in St. Louis and at all other downstream sampling points including New Orleans -- a distance of 1,000 miles. Normal water treatment processes are not very effective in removing organic contaminants.

To better visualize the appearance of organic contaminants, photographs of some concentrates are illustrated in Figs. 1-3 (pages 42, 43, and 44).

Finding the Cause of Damages

In seeking for the chemical causes of water damage, what approaches can the chemist use? One of the major water damages caused by organic contaminants is taste and odor in drinking water. Some are of natural origin, some of industrial origin. If tastes and odors are reported, what tests can be used to seek the cause?

1. Threshold and qualitative taste and odor tests on the water are used first. Here the sense of smell and taste are applied for direct measurement. The senses are sensitive, if not precise. If the problem is not too complex, an experienced observer may gain leads as to possible causes of the trouble by this procedure. Most taste and odor problems are very subtle and usually are caused by a mixture of materials. Separation techniques are required to further isolate odor-bearing materials.

2. Specific contaminants such as the phenols can be tested for by direct colorimetric means. In addition, suitable paper chromatographic tests permit separation and identification of such compounds as phenol, cresol and certain of their substituted compounds. A combination of chemical and taste and odor tests will usually establish the presence or absence of phenols.

Fig. 1 - The Appearance of Water When Concentrated 1000 Fold.

Sixty liters of each water sample were concentrated by means of a vacuum evaporator to 60 ml. The salts were filtered off and the liquid was bottled. Color ranged from clear to deep yellow.

Labels identify: 1. Distilled water; 2. Well water serving Clearmont County, Ohio; 3. Cincinnati tap water; 4. Cincinnati tap water after passing through activated carbon filter before concentration; and 5. Untreated Ohio River water at Cincinnati.

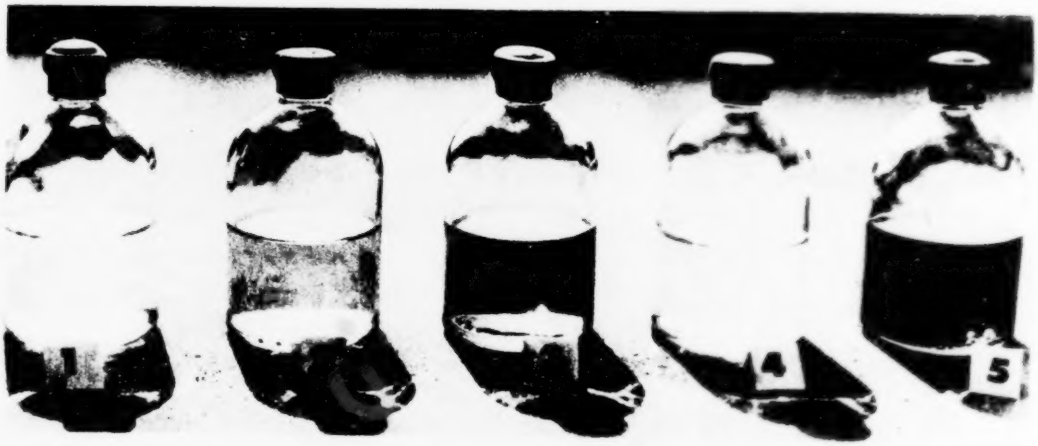


Fig. 2 - Carbon Filter - Chloroform Extracts from Water Samples.

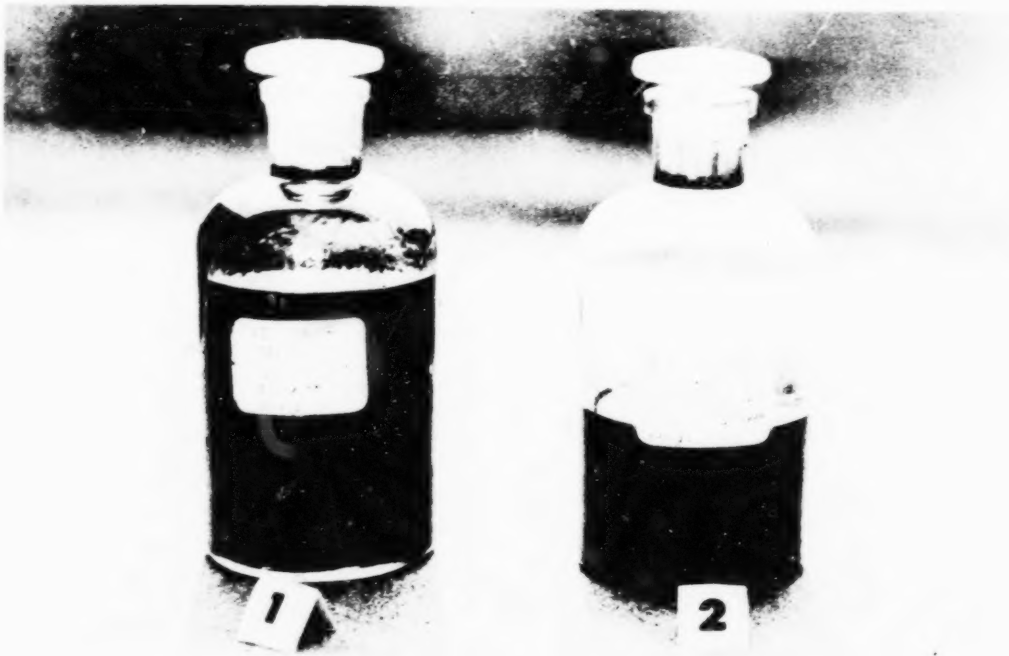
The chloroform extractables resulting from passing 5000 gallons of river water through a carbon filter are illustrated. Sampling stations were selected to show the gradation of these types of organic contaminants from clean water to those seriously contaminated with industrial wastes.

No.	Location		Concentration in parts per billion	Pollutional Situation
	River	City		
1.	Columbia	Bonneville Dam	24	Clean water sewage and moderate.
2.	Missouri	St. Louis	47	Amounts of industrial waste.
3.	Ohio	Cincinnati	165	Sewage and industrial waste.
4.	Ohio	Cincinnati	200	Illustrating a level of 200 ppb. proposed as a standard for these contaminants in drinking water.
5.	Kanawha	Winfield, W. Va.	457	Severe pollution from chemical wastes.

Fig. 3 - Raw and Treated Water Carbon Filter - Chloroform Extracts.

Fifty thousand gallons of water from a polluted source were passed through a carbon filter, and the same amount of tap water was passed through a similar filter.

Water treatment consisted of high pressure aeration, coagulation, treatment with 10 to 20 ppm. of activated carbon, sand filtration and chlorination. It can be seen that this extraordinary treatment effort reduced the concentration of extractable contaminants by only one-half. Routine treatment often appears to remove little or none of the contaminants.



3. The carbon filter technique, developed at the Sanitary Engineering Center and used during the past ten years, offers one approach in attempting to recover and identify trace amounts of potentially troublesome organics in water, including odorous compounds.

The organic contaminants are concentrated by passing 3,000 to 5,000 gallons of water through a filter containing activated carbon. The adsorbed organics are extracted from the carbon with chloroform followed by a second extraction with alcohol. Removal of the solvents leaves organic residues which are ready for study.

The chloroform extract is separated into broad chemical groups, and these groups are further examined by means of infrared, column and gas chromatographic procedures and classical methods of organic chemistry.

Odor tests on separated fractions may reveal the chemical groups that are responsible for taste and odor. As in any determination, it is important that every effort be made to correlate laboratory and field findings. In many cases the actual cause of taste and odor escapes even this procedure. The technique may miss materials of high volatility or those so fragile as to be destroyed in the testing. It is most valuable in picking up stable chemicals of industrial origin, particularly synthetics. Organic components other than odor-bearing materials are also adsorbed, but identification is difficult in most instances.

Large-scale carbon filter and extraction apparatus has been acquired for more rapid sampling and for processing of organics from several hundred thousand gallons of water. The contaminants are recovered in amounts that permit examination for toxic or other physiological properties. Such tests are now underway at the National Institutes of Health and the Sanitary Engineering Center.

4. Direct liquid-liquid extraction procedures are quite useful and are simpler than the carbon filter technique. However, a higher concentration of the pollutant is usually necessary for such procedures to work effectively. The extracts recovered are handled much like those from the carbon filter.

5. Concentration by distillation is sometimes useful. For example, in water from a well, polluted with odorous chemicals, concentration of the contaminants was achieved through distillation. Firm identification of the materials, which proved to be pyridine and other nitrogen bases, was by infrared and X-ray diffraction patterns of the chloroplatinate derivatives.

Effect of Organic Contaminants

Fish kills are often caused by organic contaminants. Low concentrations of modern insecticides, particularly the chlorinated hydrocarbon group, can cause such kills. Endrin at concentrations of 1 ppb will kill fish. If investigation reveals that one of these compounds has been used in the area, a sensitive technique for detection of that particular compound may be selected.

Where the contaminant is unknown, a technique to recover several compounds is needed. Certain chlorinated insecticides can be recovered and identified by a procedure based on concentration by the carbon filter, column chromatography, and infrared spectrometry. Thus far, the procedure has been applied to DDT, DDD, chlordane, methoxychlor, endrin, dieldrin, and aldrin. It is capable of measuring contamination from these insecticides in concentrations of micrograms per liter.

Monitoring by such methods permits detection of contaminants before damage is done. DDT in concentrations of 5 to 20 ppb has been found in five major water sources. Toxaphene, however, is not readily measured by any technique. If organic phosphorus insecticides are sought, technqiues are much different. Here the method involves *in vivo* inactivation of fish brain acetylcholinesterase and determination of the residual AChE activity.

Effect of Synthetic Detergents

Foaming of water upon being drawn from the tap or upon agitation is becoming common in some locations. The principal cause is contamination of the water with synthetic detergents of the anionic type. Alkyl benzene sulfonate (ABS) is the principal surfactant implicated, and 1 ppm of it will usually cause foam.

Where septic tanks and shallow wells are located close together in porous soils, detergents from the tank effluent find their way into the well. The detergent serves as an indicator that sewage pollution is occuring. The detergent itself is not known to be of any physiological significance. However, foam on drinking water is undesirable from an aesthetic standpoint.

The presumptive presence of ABS in concentrations of 0.1 ppm or more can be detected by a procedure using methylene blue as a complexing agent. Extraction of the complex with an organic solvent and spectrophotometric determination against a standard ABS completes the test. The method has been studied extensively but still has some defects. A much longer but more certain procedure involves several extraction steps for purification of the sample, followed by infrared analysis. Some foams are caused by natural substances which cannot be readily identified.

Taste and Odor from Gasoline

Gasoline in water can cause taste and odor if the hydrocarbon is present in amounts of a few micrograms per liter. Many cases of suspected gasoline contamination are occurring in ground waters.

Our techniques for recovering and identifying these small amounts of gasoline are weak. Mass spectrometric methods have been used but are not suitable for every-day use by most laboratories. It seems reasonable that methods for measuring gasoline using the newer sensitive gas chromatographic instruments can be developed.

Polycyclic Compounds in Water

Polycyclic aromatic hydrocarbons may reasonably be expected to be polluting river waters, although at very low concentrations. The materials have been isolated from sewage effluents and industrial wastes, and some identifications have been made in carbon filter extracts from the water.

The solubilities of some polycyclics in water are:

Benzpyrene	4	micrograms/liter
Chrysene	1.5	micrograms/liter
Anthracene	75	micrograms/liter
Phananthrene	1600	micrograms/liter

Solubility figures offer a guide as to what concentrations may be present. However, other solubilizing materials in the water may increase the amounts recoverable.

We have under development an analytical procedure which we believe will permit the determination of polycyclic hydrocarbons in water at concentrations of a few parts per trillion. The procedure involves adsorption on carbon, desorption with an organic solvent, purification and separation by column chromatography, and final determination by UV spectra.

The development and testing of such a procedure is a long process. It is difficult to say how much time and effort should go into developing such a procedure. However, if materials of known physiological significance are present, even at concentrations below any danger point, we can scarcely afford not to measure them to the best of our ability.

General Survey of Organics

The organic composition of our oldest and most abundant waste, domestic sewage, is only partially known. Painter and Viney (1) have attempted to describe the composition of domestic sewage. By analyzing for carbohydrates, amino acids, higher fatty acids, soluble acids, esters, anionic surface-active agents, amino sugars, amide and creatinine; and then assuming these to contain organic carbon in the same proportion as selected representative compounds (e.g., glucose for carbohydrates), the organic carbon that could be accounted for was totaled. This was compared with the total organic carbon measured. By this procedure 75% of the organic carbon was accounted for.

Bunch (2), working with the soluble components of sewage effluents, found that 65% of the organic constituents remained unclassified. The total organic matter is difficult to measure with certainty. The C.O.D. test gives part of the answer but some organics are not measured. Volatile content measured by igniting at 600° C gives information on the amount of organics, but the reading is likely to be higher than the actual amount present because of the difficulty of accounting for volatilization of certain inorganics and loss of water of crystallization.

In studies utilizing the carbon filter technique, a variety of chemical contaminants have been identified (3, 4, 5). Some materials found in river and drinking waters are DDT, aldrin, orthonitrochlorobenzene, tetralin, naphthalene, chloroethyl ether, acetophenone, diphenyl ether, pyridine and other nitrogen bases; phenols of various kinds, nitriles, acidic materials; miscellaneous hydrocarbons, including substituted benzene compounds, kerosene, synthetic detergents, aldehydes, ketones and alcohols. Many other compounds that are undoubtedly present remain unidentified.

Very recently our analytical procedures have been upgraded by developments in the field of gas chromatography. Chloroform extracts from the carbon filter samples are steam distilled, separations are made by the usual solubility procedures, fine separation of the fractions is achieved by the gas chromatographic method followed by collection of the separated samples and infrared analysis. Identifications that are not possible with less refined methods can be made by these means.

The individual compounds named have been present in water in concentrations of less than 1 milligram per liter, but the combined amounts of organics in rivers and even in some drinking waters has been as high as 3 mg/l. It is estimated that citizens of Cape Girardeau, Missouri, have had at least a year's exposure to a dosage of 75 micrograms per day of orthonitrochlorobenzene. This chemical was in a total mixture of organics (as measured by the carbon filter) which constituted an exposure of 800 to 1,000 micrograms per day. In at least one instance, water consumers are exposed to 1,500 to 2,000 micrograms per day of mixed organic chemicals principally of industrial origin. Can such concentrations affect health? Even if health is not a consideration, other damages from the materials require a close look at what can be permitted in water.

The Need for More and Better Information

Organic contaminants are already causing the deterioration of water quality. Some of these contaminants can be measured by suitable techniques (6); some cannot. Knowledge of the kinds and quantities of chemicals in our industrial effluents, sewage, rivers, and drinking waters is important if we are to find the causes of water damages now occurring and to assess possible physiological hazards. We need to extend our knowledge of the total role of organic contaminants in water by the following:

1. The problem of identification and measurement should be attacked more vigorously. Techniques already available have not been applied to the water problem. New techniques will need to be developed and automation should be a goal. Research groups in universities, government, industry and other establishments can best do this.

2. The possible human physiological effects from organic contaminants should be evaluated. What concentrations of what chemicals are significant?

3. A more complete description by industrial groups may be required of what is discharged into the water and what the possible effects on water quality will be. Process industries are in the best position to evaluate their wastes from a technical standpoint.

4. A strong research program should be sponsored or preferably carried out by the water industry, as the final barrier between contamination and the consumer, to develop new methods of coping with our modern contaminants once they reach the water intake.

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OCCURRENCE OF TRACE ELEMENTS IN WATER

By

W. H. Durum

In the past few years important breakthroughs have been made in the study of trace (or minor) elements in fresh waters. Once considered an area of chemical and geochemical curiosity, but having limited practical application, the study of minor elements is now an integral part of serious investigations of water chemistry.

High on the list of accomplishments is improved methodology which has provided the tools for quantitative measurement of the elements currently assessed as the most significant. Of particular importance is the refinement of techniques for the spectrographic determination of minor elements in water that provide sensitive, practical, and reproducible results.

Source of Dissolved Substances in Water

The principal bulk of dissolved constituents in natural water is contributed by the soluble products of chemical weathering and soil formation. Thus the factors that control chemical weathering also determine the amounts of trace elements available for solution. Some of the chemical factors affecting release of trace elements from primary materials and their stability in solution are solubility, pH, adsorption characteristics, hydration, coprecipitation, colloidal dispersion, and complexes.

An important but generally neglected source of trace elements in water is vegetation. Many plants selectively concentrate trace elements, as well as major elements, that become available during the decay or transportation of the plant. The decay and solution of the organic matter may form metal-organic complexes such as agents causing the stabilization of ferric iron in swamp waters.

Man's activity in mining and manufacturing is an important unmeasured source of trace elements in natural water.

Three Field Studies and Three Analytical Methods

This paper is concerned principally with recent trace-element studies of water by the U. S. Geological Survey.

Three different spectrographic methods have been used in conducting three widely different field programs, each having its own objectives. The three-prong approach shortened the time table required for selecting adequate tools for each program and thus provided greater breadth of experience in a relatively short period of investigation.

Dissolved trace elements as reported herein are those elements that collectively do not generally exceed one milligram per liter in natural water.

Studies of Principal Rivers

One program (Durum, Heidel, and Tison, 1960) updates basic information reported in Clarke's "Data of Geochemistry," and provides a reservoir of current background data on rates of continental chemical losses to the oceans. An important product of the study of ocean-borne chemicals in principal rivers has been new and meaningful quantitative data for dissolved trace elements.

Samples were obtained in the lower reaches of the river just above tide and were prepared in the field by a unique and simple filter system. Trace elements were preserved by the addition of concentrated nitric acid. Duplicate residue analyses were then made on the spectrograph using the regions of the spectrum (2250-4750A and 6250 to 8750A) in which most of the metals are found. The application of emission spectrography (Haffty, 1960) proved to be rapid and reliable in the determination of quantitative concentrations of trace elements in 65 principal rivers of the world.

The method by Joseph Haffty (1960), an adaptation and refinement of the Braidech and Emery (1935) carbon-arc procedure, facilitates quantitative analysis of 24 trace elements which are commonly found in natural waters. Major elements are determined chemically and are not reported in this paper.

Barium and Strontium Content

Let us look briefly at a few data. Preliminary results for the United States and Canada (Table I) show that of the alkaline earth metals, barium occurs in all waters examined in concentrations ranging up to about 150 micrograms per liter, and strontium up to about 800 micrograms per liter. Undoubtedly these values are exceeded at very low flows although barium concentrations seem to be independent of the rate of water discharge.

Generally, there is no well-defined relationship between strontium content and streamflow. However, samples from the Mississippi and Atchafalaya Rivers do show an increasing trend in strontium content and in the ratio of strontium to barium as discharge decreased, shown in Fig. 1. Also, the ratios of calcium to strontium and calcium to barium tended to increase with discharge in the two river waters, as in Fig. 2. The alkaline earths also increased with an increase in dissolved solids for the Mississippi River, as in Fig. 3, which follows from observations in Fig. 1.

Of the alkali metals, cesium, rubidium, and lithium occur in lower levels of detection or not at all, suggesting that the alkali metals in principal rivers are strongly sorbed by soils and rocks upstream. Zinc and cobalt rarely occur in measurable quantities. Chromium, nickel, copper, lead, and boron were found in most waters, but no relation to streamflow is evident. Titanium occurs in most waters, and at times exceeds 70 micrograms per liter. Molybdenum and vanadium were detected in many samples in low concentrations. Values for copper, manganese, and boron were of the same order as those reported by Konovalov (1959) for major rivers of the USSR.

There emerges, from the data in Table I, something of a pattern of occurrence or absence of a particular trace element in waters from a particular large drainage network. Hydrologic and geologic implications are already evident. Waters in the lower reaches of these principal rivers are a meld of many upstream tributaries. Probably much greater divergence in the kinds and amo amounts of trace elements will be found within the drainage networks than is evident from data for the main stem. This must be kept in mind in assessing current results.

Strontium Occurrence in U. S. Waters

The second approach was used by Skougstadt and Horr (1960) in establishing the general occurrence of strontium in fresh water of the United States. National interest has been focused on strontium and its occurrence and distribution in nature because of health hazards attributed to the radioactive isotope, strontium-90. Skougstadt used a Beckman Model DU spectrophotometer with flame attachment for strontium concentrations more than about 0.2 ppm.

Durum: Table I

Spectrographic analyses for minor elements in waters from principal rivers of the world

[Spectrographic analysis in micrograms per liter except as indicated] a

Date of collection	Discharge (cfs)	Silver (Ag)	Aluminum (Al)	Boron (B)	Barium (Ba)	Beryllium (Be)	Cobalt (Co)	Chromium (Cr)	Cesium (Cs)	Copper (Cu)	Iron (Fe)	Germanium (Ge)	Lithium (Li)	Manganese (Mn)	Molybdenum (Mo)	Nickel (Ni)	Phosphorus (P)	Lead (Pb)	Rubidium (Rb)	Silicon (Si)	Tin (Sn)	Strontium (Sr)	Titanium (Ti)	Vanadium (V)	Zinc (Zn)	Zirconium (Zr)
(47) St. Lawrence River at water works plant at Levis, Quebec, Canada																										
Aug. 25, 1958	342,000	0.94	276	13	30		0	12		4.3	580		0.41	21	1.7	13	0	3.7	1.4			66	21	--	0	
Sept. 3	340,000	0	186	6.3	26		0	9.6		4.7	257		.40	6.7	2.4	11	0	4.9	1.4			92	6.6	--	0	
Sept. 24 c	342,000	<.14	1,050	11	46		4.0	23		59	1,190		.60	30	2.6	22	173	55	2.9			78	50	--	<144	
Dec. 3	348,000	<.14	828	11	36	0	0	5.8		4.7	718		1.1	18	1.1	13	0	3.2	1.4			86	51	<4.1	0	
Apr. 20, 1959 c	551,000	<.11	--	7.8	39		<1.1	6.8		--	--		.27	69	<.34	13	<112	7.3	<1.1			45	--	<3.6	<112	
Apr. 23 c	550,000	<.11	--	5.8	40		<1.1	9.7		--	--		.36	60	.37	12	112	3.6	<1.1			58	--	3.9	0	
June 16 c	404,000	0	206	4.1	29		0	7.0		8.1	288		.55	16	.60	16	0	3.6	1.5		0	69	12	0	0	
(48) Columbia River below the Dalles Dam, about 3 miles above the Dalles, Oreg., U.S.A.																										
June 11, 1958		0.09	--	11	33		--	9		3	--		--	--	0.x	x.0	--	1.2	--			30	2.9		--	
Dec. 1		.15	238	3.9	48		0	18		3.8	280		3.9	14	2.1	10	0	5.0	1.4			112	5.2		0	
Sept. 8, 1959		<.11	82	5.0	44		<1.1	20		27	131		.59	5.5	1.0	36	0	3.4	<1.1			60	2.1	4.0	0	
(49) Yukon River at Mountain Village, Alaska, U.S.A.																										
May 28, 1958	d450,000	0.31		11	26		0.x	2.3		6.3						x.0	x00.	1.5				15	16			
Jan. 7, 1959	d30,000	<.2	82	13	109		0	7.0		2.5	1,130		2.0	181	1.2	17	<195	8.6	0			129	<2.0		0	
(50) Fraser River at Mission City, British Columbia, Canada																										
July 1, 1958	255,000	0	327	2.6	17		<1.9	2.9		1.6	1,180		0.5	26	0	5.0	<62	<0.62	2.6			16	17	0	0	
Oct. 1	166,000	<.07	526	11	18		1.9	6.0		2.5	635		.18	32	0	12	73	1.8	.95			18	16	--	0	
Feb. 12, 1959	37,100	0	261	2.6	20		0	23		3.0	363		.40	23	.79	13	<79	3.9	.79			40	18	0	0	
June 12	275,000	0	51	1.4	14		0	2.8		.83	52		1.3	3.0	<.21	2.6	0	1.3	.90		0	23	2.6	0	0	
(51) Sacramento River at tower bridge on Capital Street, at Sacramento, Calif., U.S.A.																										
May 1, 1958	b49,900	--	--	22	9		--	0.7		1.4	--		--	--	--	0.x	--	--	--			6.3	3.1	--	--	
Nov. 25	b13,300	<0.086	60	10	31		0	4.4		2.9	112		2.1	6.3	<0.43	7.1	0	4.5	1.0			46	<.86	<86	--	
May 1, 1959	b11,800	0	286	9.8	25		<3.3	2.4		7.0	1,210		.77	50	0	6.6	<110	4.2	1.1			15	14	0	<110	
Sept. 16	b9,900	<.16	1,110	25	56		<1.6	7.0		14	764		.66	34	<.47	20	0	4.4	<1.6			61	106	6.7	0	
(52) Mobile River at Mt. Vernon Landing, Ala., U.S.A.																										
Dec. 16, 1958	b24,000	0.13	186	3.3	75		0	2.0		3.5	392		1.7	41	0	6.9	<98	15	1.3			68	3.6	--	0	
Apr. 1, 1959	b92,000	.28	818	17	47	0	0	3.4		5.6	735		2.4	20	.28	6.1	<93	3.1	3.9			39	38	4.0	0	
Aug. 25	b12,000	<.089	84	1.6	64		<.89	6.8		28	125		.14	7.4	.35	29	0	1.5	<.89			59	2.7	0	0	
Sept. 30	b15,500	<.085	289	4.3	52		0	1.9		2.7	204		.70	6.7	.27	6.8	0	1.2	2.6		1.4	51	12	0	0	
(53) Colorado River on bridge on U. S. Highway 80, at Yuma, Ariz., U.S.A.																										
Sept. 16, 1958	b1,020	1.0	12	52	152		0	24		8.8	160		35	21	6.9	30	0	<8.0	<8.0			802	<8.0		0	
Jan. 20, 1960		0	153	34	128	0	0	10		8.5	111		37	37	6.5	0	0	16	0		0	715		0	0	

a For samples obtained in 1959, looked for but generally not detected: As, Au, Be, Bi, Cd, Ce, Cs, Dy, Er, Eu, Ga, Gd, Ge, Hf, Hg, Ho, In, Ir, La, Lu, Nb, Nd, Os, Pb, Pr, Pt, Re, Rh, Ru, Sb, Sc, Sm, Sn, Ta, Tb, Te, Th, Tl, Tm, U, V, W, Y, Yb, and Zr.

b Daily mean discharge.

c At Quebec.

d Estimated discharge at Kaltag.

< Detected, but less than figure indicated.

x Refers to semi-quantitative determination in the digit order shown.

Durum: Table I (Continued)

Spectrographic analyses for minor elements in waters from principal rivers of the world

[Spectrographic analysis in micrograms per liter except as indicated] a

Date of collection	Discharge (cfs)	Silver (Ag)	Aluminum (Al)	Boron (B)	Barium (Ba)	Beryllium (Be)	Cobalt (Co)	Chromium (Cr)	Cesium (Cs)	Copper (Cu)	Iron (Fe)	Germanium (Ge)	Lithium (Li)	Manganese (Mn)	Molybdenum (Mo)	Nickel (Ni)	Phosphorus (P)	Lead (Pb)	Rubidium (Rb)	Silicon (Si)	Tin (Sn)	Strontium (Sr)	Titanium (Ti)	Vanadium (V)	Zinc (Zn)	Zirconium (Zr)
(54) Hudson River at Ford Motor Company power plant, at Green Island, N. Y., U.S.A.																										
Oct. 29, 1958	23,000	0.15	304	9.0	28		0	30		8.6	207		2.2	35	0	12	0	2.9	1.9			106	<1.4	--	0	
Jan. 22, 1959	63,000	.18	775	15	46	0	<1.3	15		15	650		1.1	150	.65	8.6	0	11	1.6			84	55	5.6	<125	
Aug. 17	1,940	.59	251	20	60		<1.1	40		44	414		.28	78	1.7	71	0	9.4	<1.1			72	10	4.3	0	
Oct. 19	3,790	<.13	225	10	49		0	19		4.5	300		.89	56	.55	5.5	0	6.6	2.1		0	106	18	3.8	0	
(55) MacKenzie River about 3 miles upstream from separation, at Arctic Red River, Northwest Territory, Canada																										
July 24, 1958	470,000	0	1,410	13	65		5.0	12		11	1,390		1.1	60	0	36	259	2.9	1.6			96	8.4		0	
Oct. 2	270,000	<0.16	440	8.6	70		0	5.7		3.9	1,030		1.0	20	0	8.2	0	6.4	<1.6			228	6.0		0	
June 9, 1959	360,000	0	869	4.7	85		0	11		4.2	706		2.5	36	.80	22	0	7.6	2.4		0	125	24	0	0	
(56) Nelson River near Amery, Manitoba, Canada (observations on Jan. 20, Apr. 9, and June 3, upstream at Kelsey power site)																										
Oct. 5, 1958	e85,000	0	226	2.5	45	--	0	5.7		2.3	287		2.9	9.6	0	5.9	0	5.3	2.3			121	9.2	--	0	
Jan. 29, 1959	e49,000	0	664	7.6	50	0	0	2.5		1.0	389		4.8	8.7	.87	4.1	0	0	0			73	32	0	0	
Mar. 9	e38,000	0	105	21	58	0	0	1.9		2.5	38		7.5	0	1.3	5.5	0	0	0			105	5.3	0	0	
Apr. 9	e36,000	0	89	3.6	56	--	0	4.7		4.2	81		8.1	<2.8	0	7.8	0	22	<2.8			86	5.9	0	0	
May 1	e75,000	0	81	7.8	57	--	0	3.4		6.0	198		3.9	3.4	0	8.8	0	4.9	<2.6			107	14	0	0	
June 3	e84,800	<.18	--	58	53	--	5.1	21		11	--		1.7	177	<.53	55	425	5.0	5.1		2.1	55	67	5.7	0	
(57) Susquehanna River at hydroelectric plant spillway at Conowingo, Md., U.S.A.																										
May 7, 1958	126,000	0.3	--	10	24		0.x	1.9		5.1	--		--	--	--	x.0	--	3.6	--			12	3.2		x00	
June 10	19,600	--	--	12	38		--	4.5		4.0	--		--	--	0.x	0.x	--	1.1	--			40	--		--	
Sept. 11	10,000	.25	27	16	25		0	3.7		5.3	31		3.5	<2.1	0	11	0	<2.1	<2.1			25	<2.1		0	
June 5, 1959	17,300	<.15	102	4.3	37	0	0	1.3		105	154		3.4	79	.54	3.9	0	7.2	<1.5			74	5.7	0	0	
(58) Churchill River east of island off Drachm Point, 8 miles south of Churchill, Manitoba, Canada																										
June 24, 1958	e40,700	<0.078	148	3.7	19		0	5.5		2.0	172		1.0	17	0	4.1	0	2.5	0.78			22	8.6	0	0	
Sept. 25	e42,500	.37	103	13	38		0	3.6		9.5	79		.95	2.6	0	5.6	0	4.0	1.1			37	3.1	--	0	
Feb. 20, 1959	e27,000	.21	183	16	49	0	0	2.8		8.5	157		2.6	4.4	.49	11	0	4.3	1.2			33	7.1	0	<122	
June 27	e61,000	<.084	134	4.5	33		0	12		2.9	185		.51	8.4	<.25	14	0	2.4	0		0	23	5.8	0	0	
(59) Apalachicola River at State Highway 20, near Blounstown, Fla., U.S.A.																										
Dec. 17, 1958	11,000	0.11	73	5.0	42		0	2.2		2.1	96		0.096	5.0	0	4.6	0	6.2	1.0			34	<0.8	--	0	
Mar. 30, 1959	54,000	<.058	2,550	11	21	<0.058	0	2.2		7.0	1,220		.70	25	<.17	2.6	<58	2.1	2.1			7.5	99	2.2	0	
Aug. 24	11,900	.098	173	5.5	29		<.75	6.9		51	150		<.075	20	.46	23	0	3.6	<.75			28	8.3	0	0	
Sept. 29	9,030	<.071	135	5.6	26		0	7.8		6.2	85		.12	4.0	.62	34	0	2.7	2.1		1.3	25	6.9	0	0	

a For samples obtained in 1959, looked for but generally not detected: As, Au, Be, Bi, Cd, Ce, Cs, Dy, Er, Eu, Ga, Gd, Ge, Hf, Hg, Ho, In, Ir, La, Lu, Nb, Nd, Os, Pb, Pr, Pt, Re, Rh, Ru, Sb, Sc, Sm, Sn, Ta, Tb, Te, Th, Tl, Tm, U, V, W, Y. Yb, and Zr.

e Approximate.

< Detected, but less than figure indicated.

x Refers to semi-quantitative determination in the digit order shown.

Durum: Table I (Continued)

Spectrographic analyses for minor elements in waters from principal rivers of the world

[Spectrographic analysis in micrograms per liter except as indicated] a

Date of collection	Discharge (cfs)	Silver (Ag)	Alum-inum (Al)	Boron (B)	Bar-ium (Ba)	Beryl-lium (Be)	Co-balt (Co)	Chrom-ium (Cr)	Cesi-um (Cs)	Cop-per (Cu)	Iron (Fe)	Ger-mani-um (Ge)	Lith-ium (Li)	Man-ga-nese (Mn)	Molyb-denum (Mo)	Nickel (Ni)	Phos-phorus (P)	Lead (Pb)	Rubid-ium (Rb)	Sili-con (Si)	Tin (Sn)	Stron-tium (Sr)	Tita-nium (Ti)	Vana-dium (V)	Zinc (Zn)	Zirco-nium (Zr)
(45) Mississippi River just above bridge on U. S. Highway 190, near Baton Rouge, La., U.S.A.																										
May 10, 1958	865,000	0.13	--	29	78		--	2.6		10	--		--	--	--	0.x	x 00.	4.0	--			34	62	--	--	
Oct. 14	262,000	.22	1,050	22	127		5.8	8.5		6.9	1,670		4.9	185	1.1	19	223	7.8	2.5			105	6.7	--	0	
Mar. 13, 1959	655,000	0	1,010	15	72		0	4.6		9.0	828		1.8	46	0	13	< 184	4.0	7.4			61	72	<5.5	0	
May 18	415,000	0	281	6.1	84		0	84		74	663		2.4	12.2	2.6	33	0	9.4	<2.6			97	12	0	0	
(46) Atchafalaya River from railroad bridge, 0.6 mile below bridge on U. S. Highway 190, at Krotz Springs, La., U.S.A.																										
May 10, 1958	323,000	--	--	14	43	0.x	0.x	2.4		3.6	--		--	--	--	0.x	--	1.1	--			11	10	--	--	
Oct. 14	130,000	0.33	725	17	132	--	5.2	30		9.9	1,230		4.5	100	<0.93	47	< 186	11	2.2			154	20	--	0	
Mar. 13, 1959	276,000	0	1,635	19	52	<.22	0	2.6		3.7	1,240		1.9	68	0	6.5	0	2.6	2.4			22	107	<6.5	0	
May 18	166,800	0	462	14	122	--	0	4.9		10	517		2.6	12	<2.7	10	0	11	<2.7			139	14	0	0	

a For samples obtained in 1959, looked for but generally not detected: As, Au, Be, Bi, Cd, Ce, Cs, Dy, Er, Eu, Ga, Gd, Ge, Hf, Hg, Ho, In, Ir, La, Lu, Nb, Nd, Os, Pb, Pr, Pt, Re, Rh, Ru, Sb, Sc, Sm, Sn, Ta, Tb, Te, Th, Tl, Tm, U, V, W, Y, Yb, and Zr.

b Daily mean discharge.

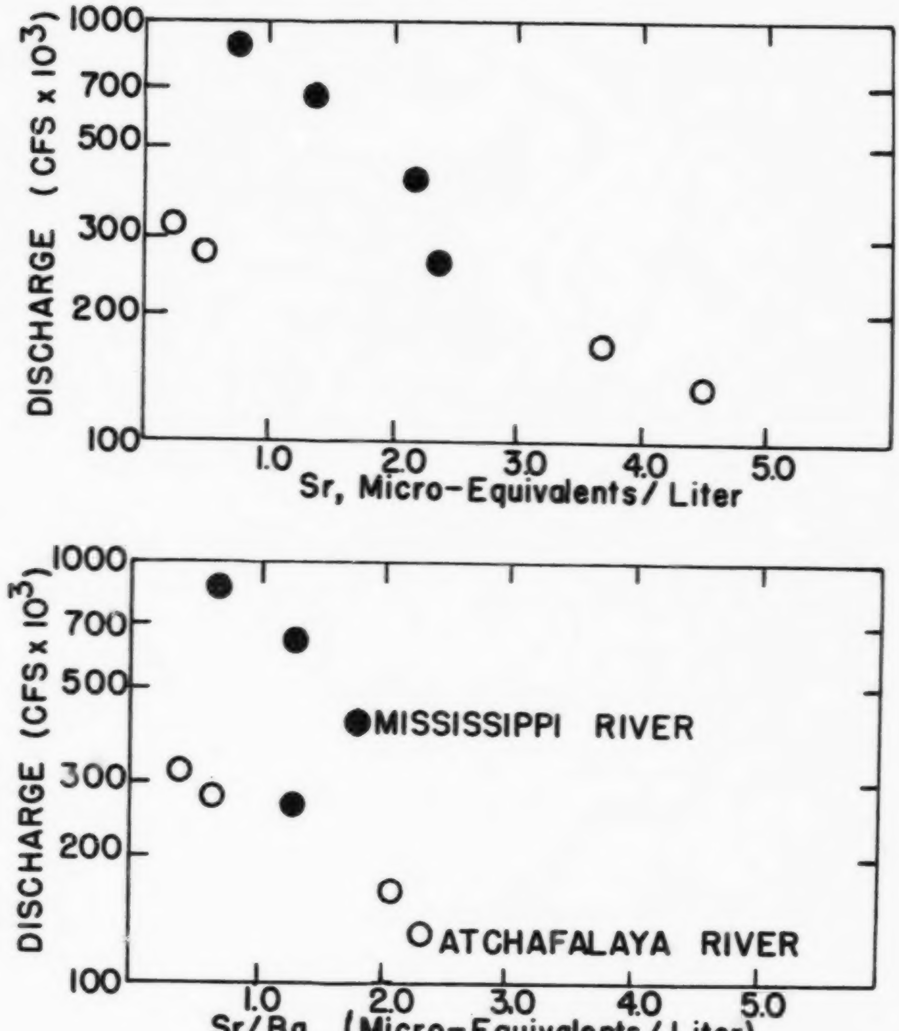
e Approximate.

< Detected, but less than figure indicated.

x Refers to semi-quantitative determination in the digit order shown.

Durum: Fig. 1

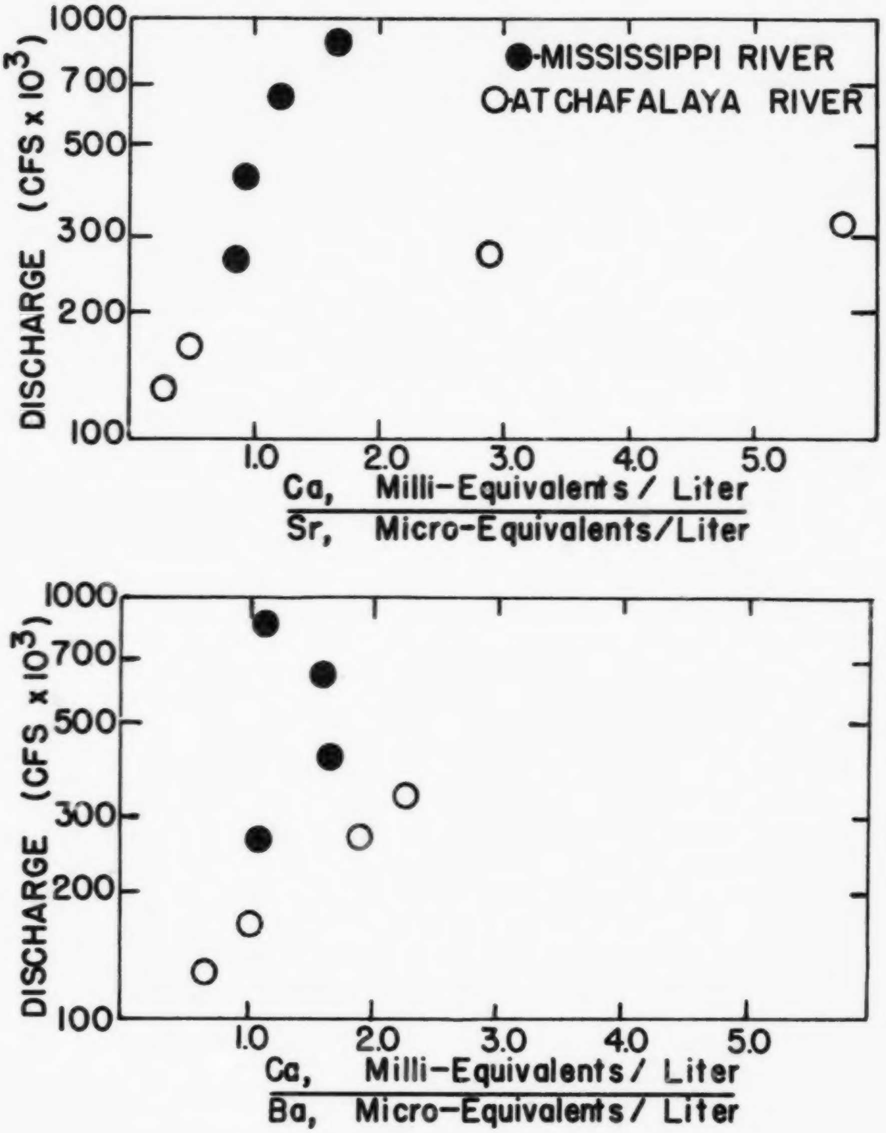
Figure 1.



Sr, Sr/Ba, AND DISCHARGE

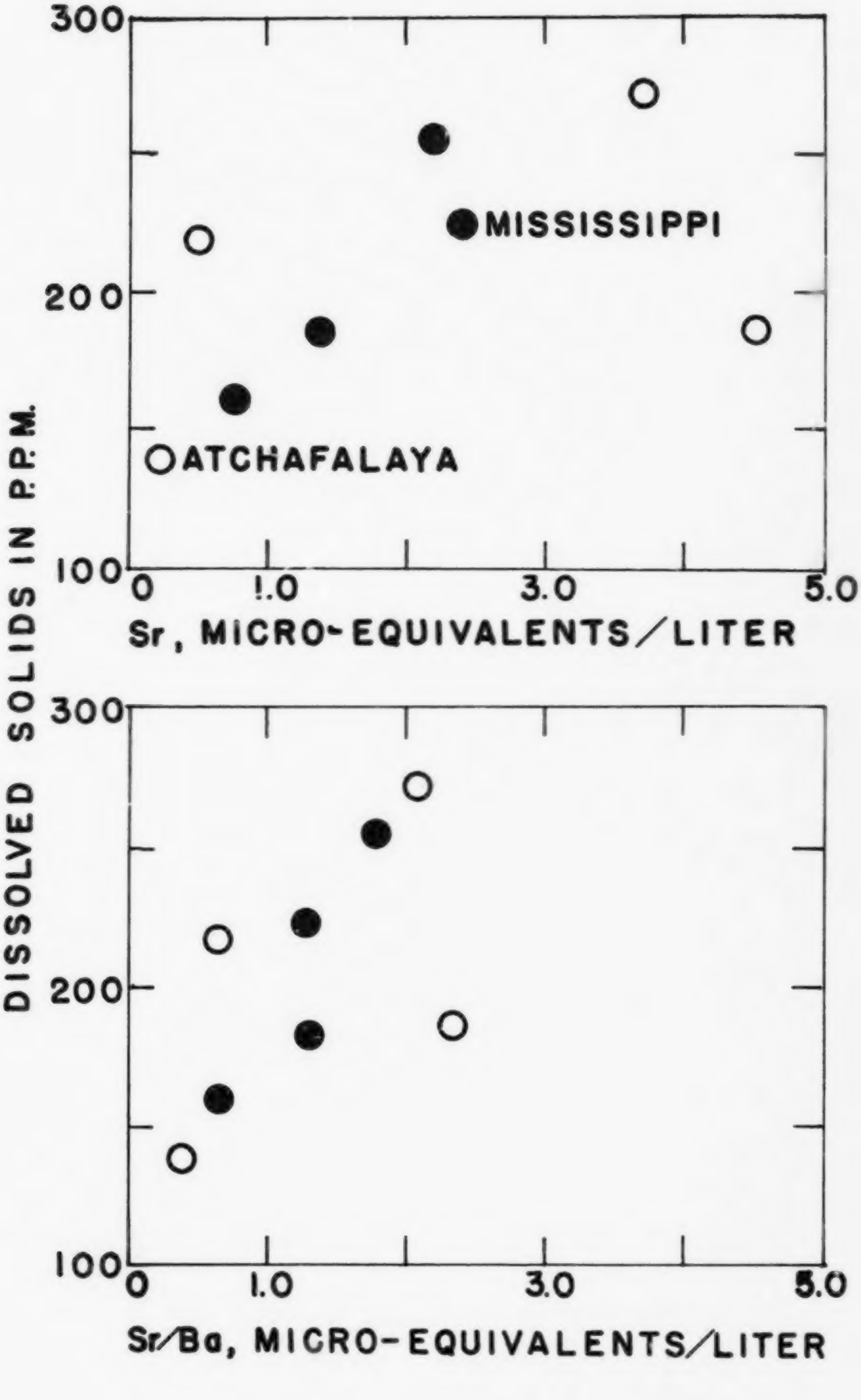
Durum: Fig. 2

Figure 2.



$\frac{Ca}{Sr}$, $\frac{Ca}{Ba}$, AND DISCHARGE

Durum: Fig. 3



Sr, Ba AND DISSOLVED SOLIDS

For greater sensitivity, a spectrographic procedure based on the copper-spark technique of Fred and Nachtrieb (1947) and Nachtrieb (1950) was used for samples containing less than about 0.2 ppm of strontium.

In the strontium study, surface water samples were obtained from about 65 places in the United States to represent, insofar as possible, the major streams and drainage areas of the country. At each site, three samples were obtained at intervals of several months to a year, to obtain water during high, low, or median flow conditions for the stream.

The results of the study are summarized by Skougstadt and Horr in Fig. 4. The regions where the strontium content of surface waters is relatively low (less than 0.50 ppm) include the Pacific Northwest, Northeastern United States, and the Central lowlands, including the lower Mississippi basin and the Western Gulf Coast area. Moderate amounts of strontium (0.50 to 1.5 ppm) are found in streams of Southeastern United States, most of the Great Plains Region, the Western Mountain and Plateau Regions, and California. Surface waters in parts of northern and western Texas and southern New Mexico and Arizona are comparatively high in strontium.

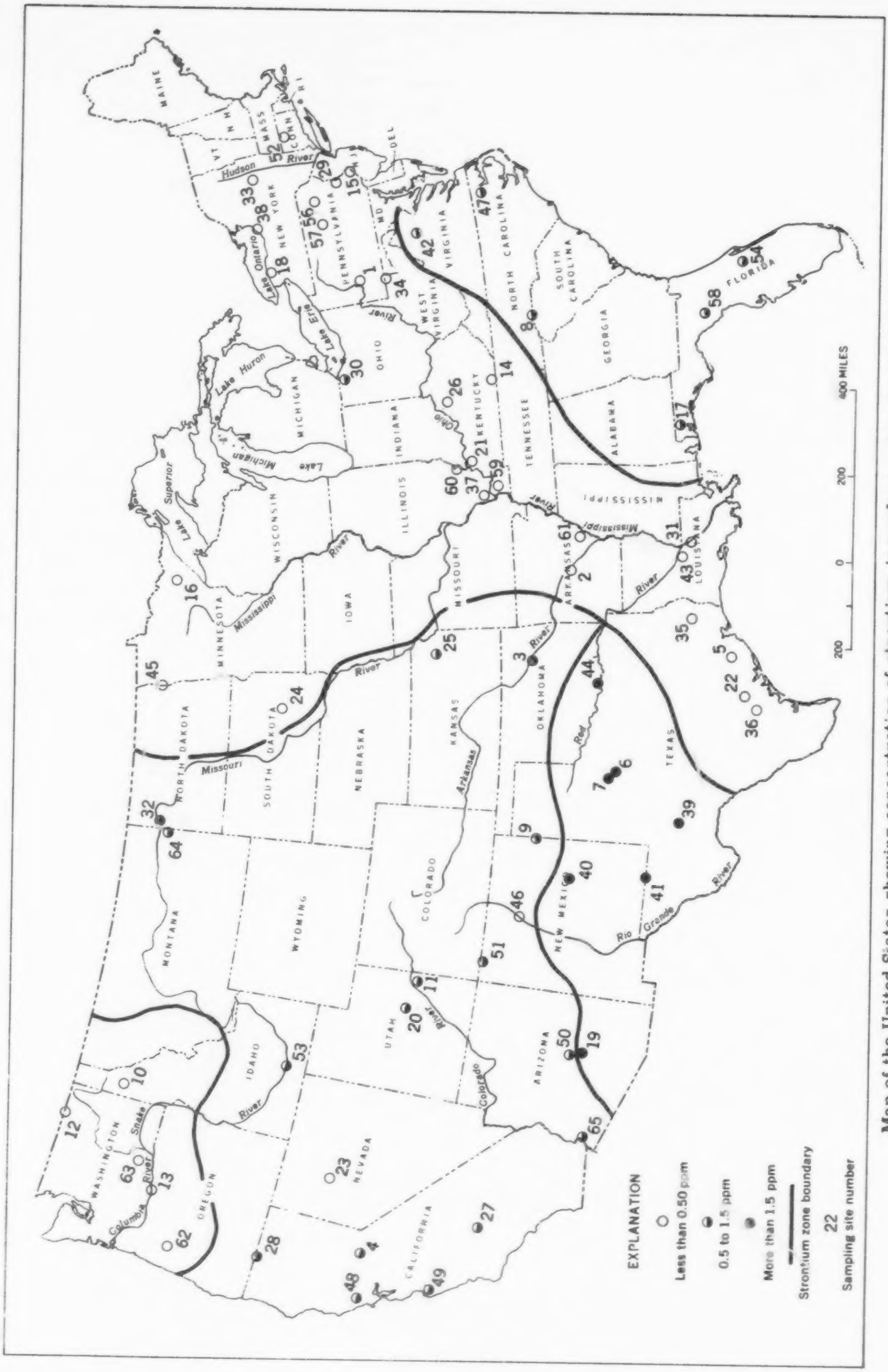
Localized geologic environment such as the extensive celestite deposits in northern Ohio gave anomalous values for strontium that do not fit the grouping in Fig. 4.

Trace Element Studies in California

A third method used by the Survey in cooperative studies with the California Department of Water Resources (Silvey, 1960) utilizes a chemical enrichment procedure prior to spectrographic analysis. This method, a refinement of the method after Heggen and Stock (1953), is particularly useful when determining trace elements in waters containing a high dissolved solid content. Data from this study are on file at Survey's Division of Water Resources, Sacramento, California.

Presence of Radioelements in Water

The natural radioelement content of water is a subject receiving wide attention and discussion at other conferences. For our purpose, however, we may consider natural uranium and radium as important trace elements, and thus emphasize the generalized studies of uranium and radium in the United States, such as that by Scott and Barker (1958).


EXPLANATION
○ Less than 0.50 ppm
◐ 0.5 to 1.5 ppm
● More than 1.5 ppm
Strontium zone boundary
22 Sampling site number
0 200 400 MILES
WASHINGTON
OREGON
CALIFORNIA
NEVADA
IDAHO
MONTANA
WYOMING
UTAH
ARIZONA
COLORADO
NEW MEXICO
NORTH DAKOTA
SOUTH DAKOTA
NEBRASKA
KANSAS
OKLAHOMA
TEXAS
MINNESOTA
IOWA
MISSOURI
ARKANSAS
LOUISIANA
WISCONSIN
ILLINOIS
MICHIGAN
INDIANA
OHIO
KENTUCKY
TENNESSEE
MISSISSIPPI
ALABAMA
GEORGIA
FLORIDA
SOUTH CAROLINA
NORTH CAROLINA
VIRGINIA
WEST VIRGINIA
MD
DEL
N J
PENNSYLVANIA
NEW YORK
VT
N H
MAINE
MASS
CONN
R I
Columbia River
Snake River
Colorado River
Rio Grande River
Arkansas River
Red River
Missouri River
Mississippi River
Ohio River
Hudson River
Lake Superior
Lake Michigan
Lake Huron
Lake Erie
Lake Ontario
12
10
63
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22
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35
43
31
2
61
59
37
60
21
26
14
17
8
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47
42
34
1
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52
29
57
56
15

Map of the United States showing concentration of strontium in surface water at selected sites

Durum: Fig. 4.

In a paper prepared for the Geneva Conference, United Nations Peaceful Uses of Atomic Energy (1958), the authors give statistic interpretation of uranium and radium data for ten geotectonic regions of the United States, which boundaries were established from tectonics, geology, physiography, and groundwater provinces. Table II taken from their report, summarizes the then available radioelement data for ground water in the United States. Additional reports are in progress.

Scott and Barker report that neither radium nor uranium concentrations correlate well with concentrations of the more common constituents of ground water, although "uranium concentrations do tend to be somewhat higher in water containing large amounts of bicarbonate ion." Table II shows the range, median, and "anomaly threshold" for radium and uranium concentrations, expressed as micromicrocuries per liter (μμc/l) and micrograms per liter (μg/l) respectively, for the ten geotectonic regions. The anomaly threshold represents the lower limit of values which can be considered likely to have come from a population different from the bulk of samples that make up the frequency distribution.

Note especially the range and median values for uranium in samples from Region VI, in West-Central United States, and for radium in samples from Region VII, the Ozark-Ouachita Mountains.

Summary

Knowledge of the occurrence of trace elements in water is one additional step in mans' continuous probe into the unknown. Only a few of many significant studies are described in this paper. However, the results make clear that a vast frontier in technical studies looms ahead for a better understanding of trace elements - the end products of many earth forces.

Durum: Table II

Table 2.--Summation of Radioelement Data

Geo-tectonic region	No. samples	Uranium (μg U/1)			Conc. factor (U) (μg U/g dis. solids)		Radium (μμc Ra/1)			Conc. factor (Ra) (μμc Ra/g dis. solids)	
		Range	Median	Anomaly thresh.	Maximum	Median	Range	Median	Anomaly thresh.	Maximum	Median
I	89	0.1-15	0.2	2.5	62	0.9	0.1-8.6	0.2	4.1	174	1.0
II	78	0.1-24	.6	10	70	4.0	0.1-3.3	.1	2.5	21	.8
III	16	0.1-18	.5	---	72	1.3	0.1-2.1	.1	---	8.4	.2
IV	6	0.2-4.3	1.1	---	10	7.4	0.1-2.6	.1	---	12	.9
V	79	0.1-8.9	.5	4.8	18	.9	0.1-22	.5	7.4	96	.7
VI	74	0.1-120	2.1	45	319	5.9	0.1-11	.3	6.3	25	.9
VII	11	0.1-0.3	.2	---	2.3	1.6	0.1-2.3	1.0	---	13	2.3
VIII	84	0.1-37	1.6	28	60	5.2	0.1-3.4	.1	2.5	12	.1
IX	12	0.1-6.1	.8	---	9.9	2	0.1-1.0	.1	---	7.6	.3
X	37	0.1-7.6	.3	7.4	18	1.3	0.1-2.5	.1	1.1	10	*

*Below detection limit (Ra 0.1μμc/1)

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Durum: Appendix

A Few Details on Methods Employed

Principal river studies -- Evaporate sample to dryness on a steam bath in fused quartz or vycor evaporating dishes. A weighed amount of the residue (12 mg) and synthetic standards mixed with a weighed amount of spectroscopically pure graphite powder (6 mg) is volatilized using a direct-current arc of 16 amperes. The resulting radiant energy is recorded on photographic plates after passing through a dispersing medium (grating), and the intensity of the spectral lines formed is measured by means of a projection comparator-microphotometer. The concentration of the elements in the unknown samples are read from working curves established by the standards arced on the same plate. By this method concentrations as low as one part per billion and fractions of a part per billion have been determined.

Emission spectrography is remarkably sensitive for some metals, such as Ag and Cu, where values of less than 0.0001 per cent of the dissolved solids can be determined. The method is less successful for some heavy metals that exhibit a spectrum not distinguished by prominent lines.

California studies -- The chemical procedure involves the use of chelating reagents (8-hydroxyquinoline, thionalide, and tannic acid) to selectively precipitate seven heavy metals (copper, lead, manganese, aluminum, chromium, zinc, and iron). The precipitate is ignited in a muffle furnace at a temperature of about 450° C. The ignited precipitate is mixed with "specpure" graphite powder. This mixture is placed in craters of graphite electrodes and subjected to a direct current arc of 6 amperes. The resulting spectrum formed is recorded on film or photographic plates. The intensities of the spectral lines are measured and the concentrations calculated.

Strontium studies -- Beckman Model DU spectrophotometer with flame attachment, an oxygen-acetylene flame, a photomultiplier tube attachment, and a spectroscopic buffer solution.

The spectrographic copper-spark method requires about 30 ml of water sample and rapidly detects concentrations of strontium to about 5 parts per billion. The probable error of an analysis is about 10 per cent of the amount present.

The usual chemical methods for the determination of strontium are not satisfactory when applied to water analysis because of the small amounts of strontium involved and the difficulty of separating a small amount of strontium from much larger amounts of calcium and magnesium normally found in natural water.

QUANTITATIVE MINERAL REQUIREMENTS

By

D. M. Hegsted

The relationship between water supplies and nutrition is not a subject which is commonly considered by nutritionists. In many nutrition surveys, designed to study food and nutrient intakes, the water supply is completely ignored although it is known that in some areas the water used may supply as much as a third of the total calcium intake. The general lack of concern of nutritionists about the composition of the water is undoubtedly based partly upon ignorance --- ignorance of the composition of water but primarily ignorance about the nutritional role of the mineral constituents commonly found in water.

The one exception to this generality is the relationship between fluoride content of water supplies and dental caries. Here the situation is reversed. There is little information on the fluoride content of foods and, as far as I know, on the relationship between fluoride content of foods and dental caries. Just why this is so is puzzling to me since one might expect that the total fluoride intake would be the dominant factor and one might expect that foods would vary as much in fluorine content as would water. Possibly it is the fact that the food supply of urban populations is so thoroughly mixed in the market place that the local conditions of food production are relatively unimportant. Nevertheless, the relationship between the fluoride content of water supplies and the incidence of dental caries is the example, par excellence, of the role the water supply may play in nutrition. Whether this may indicate similar future possibilities is, of course, a matter of conjecture.

For many years there has been a suspected relationship between water supplies and endemic goiter or iodine deficiency. As far as I am aware, the evidence is that there have been changes in the incidence of colloid goiter in various areas which may, in some cases, have coincided with a change in the water supply. I am told that in Bogota, Colombia, goiter was extremely common up to and during the first part of this century (as it still is in many other areas of Colombia). The introduction of the municipal water supply is supposed by some to be related, at least time-wise, to the decrease and almost disappearance of this condition.

Whatever the reasons may be, Bogota now has much less goiter than many other surrounding areas of similar geography. Thousands of water analyses have been made to see whether any relationship between water composition and goiter could be uncovered, so far without success. Of course, the development of an urban society is accompanied by many changes including the importation of food from further and further afield. Whether this might explain a changing prevalence of goiter is unknown. Furthermore, good data upon the actual iodine requirement of man under different environmental conditions is practically non-existent.

Variable Diet Composition

The point I should like to make in this regard is that there is now considerable evidence that the nutritional requirements of individuals is not simply a matter of age, sex, weight and activity. These are the parameters that have been traditionally used in the establishment of nutritional standards. In several situations it is known that the basic composition of the diet may also be an important variable. People living upon the low fat diets typical of much of the world, for example, may well have dietary requirements for vitamins and minerals which may be quite different from the people in the U. S. living upon high fat diets. Work is only beginning to determine the magnitude of these effects. If they are sufficiently large, then it will follow that an estimation of the adequacy of a diet cannot be determined by a knowledge of the composition of the diet alone.

Utilizing the situation of endemic goiter as an example, it has been claimed that endemic goiter is not basically an iodine deficiency since there is not a good correlation between iodine intake and the prevalence of the disease. Actually there is insufficient data on the iodine content of foods to decide how important this argument may be. However, my interpretation would be that, should this be shown to be true, it would be an indication of the differences in iodine requirement in different areas. Since the utilization of iodized salt has been uniformly successful in the control of endemic goiter, this seems logical.

Calcium Balance in the Diet

The job of those in nutrition is to quantitate the nutritional requirements and then see that these requirements are met. As I have indicated, there is reason to believe that these are not constant parameters and cannot be uniformly applied. According to many people, calcium deficiency is the most common nutritional deficiency in the

United States. This conclusion is reached by comparing the calcium intake in the population with the Recommended Dietary Allowance of the Food and Nutrition Board. The allowance, which is 0.8 grams per day for an adult and proportionately more for children, is based upon calcium balance data. It is assumed that in the adult, the calcium requirement is the amount required in the diet to equal the calcium excretion. In the average U. S. adult this is about 10 mg per kg per day.

The rather strange and disturbing fact is that if calcium deficiency is common in the United States, it should be extremely severe in most of the world. Although at least 90 percent of the world's population does not consume as much calcium as does the U. S. population, clinical evidence of calcium deficiency is extremely rare. The studies which we did in Peru indicated the reason for the inconsistency (1). When calcium balance studies were done on men who were accustomed to consuming a low calcium diet, it was apparent that calcium balance was maintained upon levels much below those needed in the U. S. (Figure 1, page 70.) The factors responsible are unknown but clearly the "calciostat" is set at a different level than in the United States.

Standards for Calcium Balance

I believe that these results demonstrate the fact that calcium balance studies cannot determine how much calcium is needed, that is, the amount desirable. People accustomed to low calcium diets throughout the world could not possibly require 0.8 grams of calcium per day in the strict sense. If they did, they would not survive. Any population must, on the average, be in balance upon the diet they customarily consume. Hence, calcium balance studies simply estimate the usual diet of the persons studied. Furthermore, although calcium is required to form the skeleton, there is no *a priori* reason for believing that more calcium is necessarily better. Thus, there is no satisfactory evidence that the diet in the U. S. is better or worse, with regard to calcium, than diets containing considerably less calcium.

It is, of course, of the utmost importance to determine the amount of each nutrient required in the various situations. This should provide the basis of the national nutrition program. If standards are set too high, as I believe we have done for calcium, it follows that there will be extensive efforts to overcome a problem which doesn't exist.

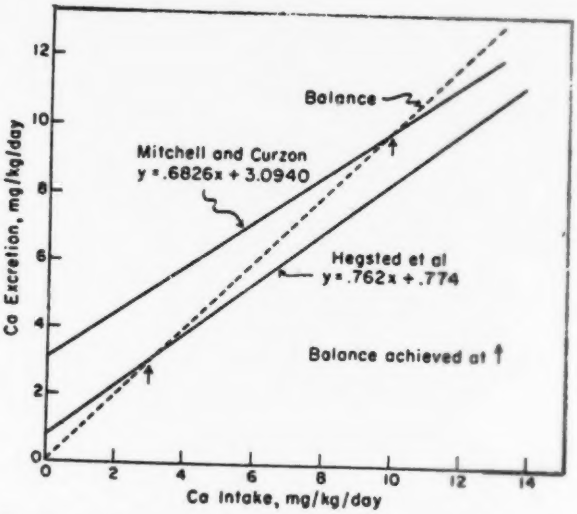

FIGURE 1.—The relation between calcium excretion and intake in the data summarized by Mitchell and Curzon (*10*) and those obtained by Hegsted *et al.* (*5*). The regression lines are essentially parallel.

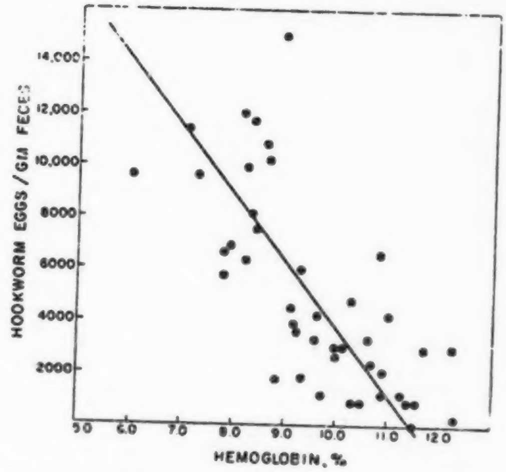

Fig. 2. The relationship between the severity of the parasitosis and the degree of anemia.

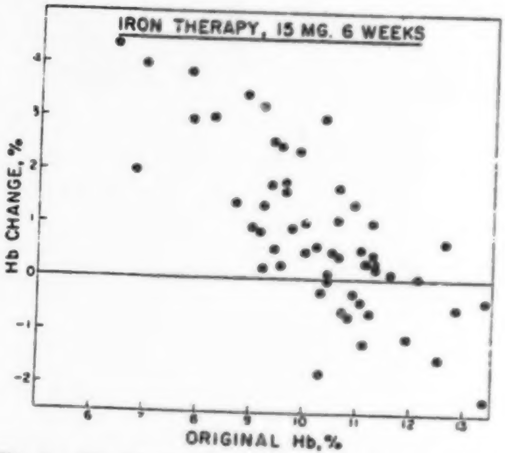

Fig. 3. The hemoglobin response of the individual children compared to their original hemoglobin level.

Fig. 1 from: Hegsted, D. M. In: Proceedings of the Symposium on Methods for Evaluating Nutritional Adequacy and Nutritional Status. Edited by H. Spector, M. S. Peterson, and T. Friedemann. National Academy of Sciences-National Research Council, p. 105, 1954.

Fig. 2 from: White, P. L., et al. In: Effects of Iron Treatment of Anemia in a Tropical Area. Am. J. Clinical Nutrition, 5: page 625, 1957.

Fig. 3 from: White, P. L., et al. In: Effects of Iron Treatment of Anemia in a Tropical Area. Am. J. Clinical Nutrition, 5: page 626, 1957.

Requirement of Iron

Another example of the need for specific information upon nutritional requirements is provided by the studies on iron requirements carried out in another part of Peru, the Amazon basin (2). This is a tropical area characterized by high rates of intestinal parasitosis and anemia. It has long been known that the anemia of hookworm disease can be treated with iron and thus is an iron deficiency anemia caused by blood loss.

From the standpoint of the public health nutritionist, it is of great importance to know how much iron is needed. If the large doses of iron currently being used by clinicians are actually required, the problem would appear to be outside the reasonability of the nutritionist, since these amounts cannot be obtained from the diet nor readily added to a diet. On the other hand, if a small dose of iron is effective, such as might be supplied by fortification of foods or by high iron foods, then the nutritionist might play a significant role.

Iron and Anemia

In the school children studied, the severity of the anemia was apparently related to the hookworm load (Figure 2, page 70). Several groups of children were given daily doses of iron, either 15, 30, or 50 milligrams daily over approximately three months. The parasitosis was not treated. The hemoglobin response after three months was approximately the same with any of the iron levels used. As would be expected, the most anemic individuals responded the most dramatically (Figure 3, page 70). No response was obtained unless the hemoglobin level was less than 10 grams per cent. The significance of this is not clear and possibly some other nutrient becomes limiting.

It does seem clear that small amounts of additional iron are quite effective in combating the severe anemia which is the major cause of disability. Dietary studies indicated that these children were receiving approximately 12 milligrams of iron per day. This is the amount recommended in the U. S. as an adequate intake. We conclude that the iron need of the children in this area, with the hookworm loads they ordinarily carry, is approximately twice the generally accepted allowance. It should be possible to plan effective nutritional campaigns on the basis of these studies. A major problem is to find a suitable carrier for the iron. Since only bread is consumed in the towns and rice is milled locally, no suitable carrier is apparent. Salt might be suitable, if a colorless iron salt could be found.

Magnesium in the Diet

Finally, I should like to mention briefly some of our recent studies on magnesium. The significance of these studies in human nutrition is entirely unknown at the present time. A number of papers have appeared recently, however, describing magnesium deficiency in many, primarily in alcoholics (3, 3a). This suggests that magnesium may not be as plentiful in our usual diets as has always been assumed, and mild degrees of magnesium deficiency may not be uncommon.

There are two specific aspects that I wish to refer to. The first is the effect in animals made hypercholesterolemic in order to produce experimental atherosclerosis. This hypercholesterolemia is produced by feeding cholesterol and cholic acid and the degree of atherosclerosis produced is, other things being constant, proportional to the serum cholesterol level (4). In early studies we concluded that the young rat required approximately 25 mg. of magnesium per 100 grams of diet to produce maximum weight gain (5). When a hypercholesterolemia is superimposed upon rats receiving a diet containing this level of magnesium, the serum magnesium falls and the signs of magnesium deficiency may develop. The magnesium in the diet must be increased several fold to maintain a normal magnesium level in the hypercholesteremic rats (6).

Effect of Magnesium Deficiency

Animals with hypercholesterolemia which are maintained upon minimal levels of magnesium develop considerably more atherosclerosis than those fed high levels of magnesium, although the serum cholesterol level is not influenced by the amount of magnesium in the diet. Thus, we conclude that a mild degree of magnesium deficiency somehow makes the wall of the arteries more susceptible to atherogenesis.

The mechanism is not yet clear, but two possibilities suggest themselves. Studies on the lipoproteins of the serum indicate that the magnesium level in the diet may affect the composition of the lipoproteins which carry the cholesterol. Also, analysis of the skin, aorta and connective tissue for hydroxyproline indicates an abnormal amount of collagen formation in magnesium deficiency. Either of these changes might possibly account for an increased susceptibility of the wall of the arteries to lipid deposition.

The major question of interest in whether these findings have any relevance to the development of atherosclerosis and coronary disease in man. This is unknown although it has been claimed that the Bantus in South Africa (7) who are relatively free of atherosclerosis and coronary heart disease, have higher serum magnesium levels than the susceptible whites living in the same general area. Little is known of the magnesium content of foods and diets, and practically nothing of the amount required. This area remains to be explored and as has been emphasized (3a), the major deterrant in this field is the lack of satisfactory analytical methods.

High Magnesium Level in the Diet

Another area of research in our department where magnesium is of interest is in relation to the production of renal calculi. Early work showed that animals fed a low pyridoxine diet (8) developed extensive renal calculi composed of calcium oxalate. More recently, Drs. Gershoff and Andrus (9) have found that if a high dietary magnesium level is supplied with the low pyridoxine diet, the production of oxalate calculi is largely prevented. Thus, it appears that when a diet limiting in pyridoxine is fed, excessive oxalate is produced. However, precipitation may not occur if the dietary magnesium level is adequate.

These studies may have application to the human disease, since dosage of human subjects - either normal subjects or oxalate stone formers - with pyridoxine usually reduces the level of urinary oxalate. By this criteria, i.e., the minimal level of oxalate formulation, it would appear that ordinary diets may not be adequate in pyridoxine. The role of either pyridoxine or magnesium as adequate therapy for oxalate stones is now being explored.

Conclusions

The conclusions I should like to make are as follows:

Firstly, rational nutrition campaigns should be based upon quantitative knowledge of the requirement of each nutrient.

Secondly, the requirement for each nutrient may vary from population to population according to the environmental conditions, and the composition of the diet is probably the major environmental factor influencing the nutritional requirements.

Finally, with the notable exception of the excellent studies on iron requirements, data on quantitative mineral requirements of man are poor and extensive investigation must be encouraged.

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EFFECTS OF TRACE AMOUNTS OF MOLYBDENUM

By

Dr. K. J. Monty

Molybdenum is recognized as an essential dietary constituent for plants and for certain microbiological systems, where it apparently plays a role in the enzymatic catalysis of nitrate reduction. The element has been demonstrated to be a constituent of the enzyme zanthine oxidase in higher animals, although attempts to demonstrate the essentiality of molybdenum in the nutrition of higher animals have led to conflicting and sometimes confusing results. It is of interest that molybdenum has been reported to be essential in the nutrition of ruminants by virtue of its apparent requirement by the microorganisms of the rumen, which carry out many important digestive functions for the host animal.

As is true of most common or essential dietary constituents, excessive dietary intake of molybdenum leads to toxic manifestations in higher animals. The adverse physiological effects of molybdenum were first noted in grazing animals. In certain localities throughout the world, the soils contain abnormally high levels of molybdenum salts. The grasses often reflect this superabundance of the metal, and animals grazing upon these high-molybdenum grasses develop a variety of abnormal physiological manifestations. The most common such manifestations are wasting (loss of weight or failure to gain weight), diarrhea, certain disorders of the central nervous system, and sympotoms of a copper deficiency (depigmentation, anemia, etc.) The latter effects may occur in spite of the diet being apparently adequate with respect to copper as determined by chemical analysis.

Physiological Response to Molybdenum

Research efforts of a number of laboratories over a span of about 20 years have led to the recognition of certain factors which affect the nature and the magnitude of the physiological response to high intake of molybdenum. When symptoms of copper deficiency appear, the animals often respond at least in part to oral admiinistration of copper salts. The addition of sulfate to the diet will in most cases completely alleviate the symptoms of molybdenum toxicity. This latter observation has made possible the practical recovery of a great deal of otherwise useless grass land.

Although corrective measures have been found for many of the agricultural problems with molybdenum toxicity, the physiological basis for the interrelationships between molybdenum, sulfate and copper have been difficult to establish. Some selected details of the mode of action of molybdenum as a toxic dietary agent are presented here to indicate the nature and the magnitude of the problem.

Molybdenum Toxicity in a Bacterium

Escherichia coli strain B was selected for these studies for two central reasons; it has been widely studied from the metabolic standpoint, and the membrane of the organism demonstrates relatively little selectivity toward inorganic ions.

When this organism was grown aerobically on a minimal medium (glucose as the only organic constituent) where sulfate was the sulfur source, the addition of molybdate to the medium resulted in a depression of growth rate. Nutritional studies revealed that the ratio of sulfate to molybdate was the factor which determined the growth rate of the organism in these experiments. Molybdate also suppressed growth when sulfite served as sulfur source, but molybdate toxicity was completely abolished by the introduction into the medium of any one of several other inorganic and organic sulfur compounds. These observations suggested that molybdate interfered specifically with the utilization of sulfate and sulfite by *E. coli*. Kinetic analysis indicated that this inhibition was of a competitive nature.

Studies with extracts of the bacterium indicated that the use of sulfate by *E. coli* involved the enzymatic synthesis of an anhydride of adenylic and sulfuric acids, and that molybdate acted as a competitive inhibitor of this synthesis. The inhibition of sulfite utilization by molybdate is thought to have an analogous explanation.

This mode of action of molybdate is shared by the selenate and tungstate ions. All three of these inhibitory ions are treated by the enzyme concerned as structural analogues of sulfate and sulfite.

In the general scheme of biology, this type of enzyme reaction is important in the synthesis of sulfur amino acids by microorganisms and by higher plants. It is also important to chemosynthetic sulfur bacteria. The food supply of higher animals and the gastro-intestinal flora of animals are obviously subject to modification by these ions through the enzymatic mechanism. The direct significance of this mechanism to the physiology of animal *per se* cannot be evaluated at present.

The Molybdenum-Copper Interrelationship in the Rat

A search for metabolic alterations in rats subjected to high intake of molybdenum revealed a substantial drop in sulfide oxidase activity in the liver. Decreases in the activity of this enzyme might permit the accumulation of sulfide and the subsequent formation of copper sulfide in the tissues of the animal. Such a sequence of events would be expected to remove copper from metabolic availability, and thus offered a hypothetical explanation for the induction of copper deficiency by molybdate.

Preliminary exploration of the hypothesis involved the creation of molybdate toxicity in rats by use of a diet high in molybdate and low in sulfate. After a few days, the sulfur amino acid cystine was added to the diet to promote the metabolic production of sulfide in the liver. Anemia was used as a convenient criterion of copper deficiency, since the synthesis of hemoglobin is dependent upon copper. The introduction of cystine in these experiments resulted in the production of severe anemias in the molybdenum animals, as predicted by the hypothesis. The appearance of the anemias could be prevented or corrected by copper therapy. Sulfate therapy also would prevent or correct the anemia.

Further support for the hypothesis was obtained through studies of the effect of nutritional factors on the level of liver sulfide oxidase activity. The introduction of molybdate to the experimental diet resulted in a rapid lowering of the enzyme activity to a steady state level of about ½ that of control animals. The subsequent addition of sulfate to the molybdate-containing diet promoted the rapid return of the enzyme activity to normal values. By contrast, the addition of cystine, which would induce the onset of anemias, produced a further drop in the level of liver sulfide oxidase activity. Thus a correlation was obtained between conditions which lower sulfide oxidase and conditions which induce anemias.

The Role of Food Restriction in Molybdate Toxicity

The daily food intake of animals is appreciably lower on molybdenum-containing diets than on control diets. When the food intake of a group of control animals was limited to equal that of animals consuming the high-molybdate diet, the same magnitude of change in the level of liver sulfide oxidase was found in both groups. Thus, the drop in the activity of the enzyme is the result not of any direct action of molybdate, but rather of the voluntary restriction of food intake characteristically observed with the high-molybdate diets.

Preliminary experiments designed to establish the reason for the voluntary restriction of food intake on the high-molybdate diets indicate that the rejection of the diet is a conditioned response, and that the sensory detection of the presence of molybdate depends upon the interaction of molybdate with unidentified constituents of the diet.

EFFECTS OF TRACE AMOUNTS OF SELENIUM

By

Klaus Schwarz

It is known to all that both too little and too much of a good thing in life has harmful effects. This is certainly true for essential trace elements: Too little, as well as too much produces disease. However, rarely, if ever, is there a true connection between the pathological aspects of a trace element deficiency and those of trace element poisoning, and if we talk about trace elements in relation to water quality, this must be kept in mind. For each of the required elements there is an optimum supply, safely guaranteeing the needed amount, on one side, while safely avoiding a damaging excess, on the other.

Trace Element Deficiency Diseases

With respect to water quality, these two opposite aspects must be considered for each essential trace element. Since it is easy to study toxic effects, but difficult to study trace element requirements, we usually know quite a bit about the poisonous effects of trace elements, but little as yet about trace element deficiency diseases. Hence, there is emphasis on the toxicology, while we are not properly and satisfactorily informed about the beneficial role of water as a source of one or the other trace factor.

Trace factors in the drinking water must naturally be included if we talk about trace element nutrition. We wish to outline here some of the fundamental aspects of trace element nutrition and trace element action, using selenium as an example. In this instance, drinking water may contribute to the required daily supply of an essential element, at least in certain areas 1/.

1/ We have found, for instance, that investigators in Oklahoma could not produce dietary liver necrosis with our diet in a strain of rats known to be susceptible to the disease. A difference in the selenium content of drinking water is suspected to be the reason for this discrepancy.

Selenium has recently been found to be essential as a dietary factor for maintenance of normal function and for prevention of fatal deficiency diseases in many species (1). The required levels are so small that they would escape our attention if classical methods of selenium determination were applied. Until this discovery was made, selenium had been known exclusively for its toxic effects and never for beneficial effects in animals or man (2). In certain areas with seleniferous soils and accumulator plants, i.e., plants which concentrate the element in their tissues, selenium can be consumed by animals in thier natural habitat at levels which are toxic and sometimes fatal.

Biological Significance of Selenium

From the description of our research on the biological significance of selenium, certain generalizations can be made pertaining to the context of this monograph; these are printed in italics. The experiments which eventually led to the detection of selenium as the essential ingredient of the so-called Factor 3 began about twenty years ago. It was then discovered that rats maintained on certain vitamin E-free diets low in sulfur amino acids would die from a severe, necrotic degeneration of the liver (Fig. 1). The composition of such a diet (3), used in our laboratory since 1949, is listed in Table 1.

Under well-controlled environmental and dietary conditions, the inbred Fischer 344 strain of rats will succumb to liver necrosis on the average after 21 days. The disease proceeds through three phases (4) and is inevitably fatal (Table 2). The initial phase of approximately one week's duration is one of depletion of the weanling animal's stores of protective factors. During this episode no signs of deficiency are detectable since the necessary amounts of protective agents are still available within the organism. Between the seventh and tenth day a transition into the latent phase takes place. This phase lasts for 10 to 14 days. During the latent phase the animal gives the appearance of normal well-being, gross pathological damage is not detectable, and even microscopic observation usually does not reveal distrubances of the liver or other tissues.

However, special methods of investigation, such as functional metabolic studies and electronmicroscopy, reveal changes. These are apparently the preliminary, predisposing conditions for the acute, terminal breakdown. Thus, electronmicroscopy (5) shows a severe distrubance of the submicroscopic, fine structure of the cell, specifically of the microsomal elongated profiles (Fig. 2). These structures, which normally contain a fine capillary space between two layers of osmophilic, RNA containing material, distend to form vacuoles. The disturbance gives a cystic, spongy appearance to the protoplasm. The changes are first seen after seven to ten days of dietary regimen.

Closely associated with this disturbance is a change in the mitochondria, which show swelling and a rounding of their structure. Since mitochondria, the "power plants" of the protoplasm, are the site of most of the oxidative reactions of the cell, it is not amazing that, concurrently with the structural change during the latent phase, we find breakdown of certain metabolic functions as well. Liver slices or liver homogenates obtained from animals during the latent phase are incapable of maintaining normal respiration in the Warburg respirometer. They show a phenomenon, designated respiratory decline, which consists of the breakdown of oxygen consumption after initial normal respiratory activity, usually after 30 to 60 minutes of incubation (6). The breakdown does not occur with normal liver tissue.

Respiratory decline is a rather specific phenomenon, characteristic of the latent phase of liver necrosis. It is preventable by the addition of certain protective agents to the diet, as well as by in vitro supplementation of the latter to deficient liver preparations.

In contrast to the normal appearance of the animal throughout the latent phase, the terminal phase is one of acute illness. It usually lasts from several hours to one or two days, and is etiologically correlated to the development of necrotic areas in the liver. The pathological change is associated with a block of portal blood flow through the organ (7). A rapid deterioration of many vital functions ensues; body temperature, rate of respiration, general reactivity and reflex responsiveness decline. The blood sugar falls rapidly, and life terminates in acute hypoglycemia (Fig. 3).

Three Phases of Deficiency Diseases

Three phases, namely, initial stage, latent phase, and terminal phase, can be said to be characteristic for deficiency diseases in general. A period of depletion is necessary, and usually there is a latent phase during which changes are minor or barely detectable, until acute, pathological damage presents itself during the final stage of the disease. Examination will reveal that the pattern is logically discernible in a large variety of deficiencies, including those of trace elements. Diagnostically, the latent phase poses particular problems. Suboptimal, but not (yet) critical dietary supply situations are very difficult to recognize and to categorize as to their etiology.

A peculiar difference between various types of yeast has played a decisive role in the discovery of Factor 3. It was found in Europe during the war, coincidental to studies on the value of such yeasts as protein sources in foods and feeds, that dried yeasts can be used as sources of protein in semipurified diets which produce necrotic liver degeneration (8). While most European yeasts applied in dietary schemes such as that of Table 1 will induce liver necrosis, American investigators using American yeasts at first failed in their attempts to reproduce the European studies and results.

British baker's yeast was imported for such dietary studies until it was discovered in 1949 that an American grown Torula yeast could be used in the same manner as the European samples (3). This American Torula is produced on waste liquor obtained as a byproduct of paper manufacturing. Addition of a few per cent of American brewer's yeast to the 30 per cent Torula yeast diet completely prevented the development of liver necrosis (9). The effect of American brewer's yeast was the conclusive proof for the existence of a theretofore not known dietary agent, designated Factor 3 in accordance with the fact that two other factors, namely cystine and vitamin E had previously been shown to be protective (see below).

In our laboratory intensive efforts were made between 1950 and 1957 to fractionate and isolate Factor 3 from brewer's yeast and other source materials. The project was exceedingly difficult. Factor 3 was refractory to isolation by virtue of its instability. It has the tendency to decompose during chemical manipulations when it is obtained in enriched form. However, using pork kidney powder as source material, pilot plant methods were worked out by which small portions of highly concentrated material could be produced consistently. Extensive operations yielded milligram quantities of semicrystalline, but still very impure material. In these fractions a peculiar odor reminiscent of horseradish was discovered under alkaline pH conditions. It was so characteristic that only a few types of compounds could be considered as its source.

Significance of Selenium

This observation led us to selenium. Determinations of selenium in the enriched, semi-crystalline Factor 3 fractions showed that there was a correlation between biopotency and selenium contents (Table 3). It was then found that not only Factor 3 from natural sources, but simple, inorganic selenium compounds afforded protection (10), a fact which proved that Factor 3 was not a vitamin. Selenium compounds turned out to belong to the most potent dietary agents known. Inorganic substances, such as selenites or selenates, prevented death from liver necrosis safely at levels of 5 - 10 µg per 100 g of diet. The 50% effective dose (ED_{50}) under our standardized test conditions was ca 2 µg of selenium per 100 g of diet (2 µg % Se), or .02 parts per million. Factor 3, i.e., the naturally occurring, organically bound selenium, seems at least 3 times as active. In other terms, supplementation of not more than .1 µg of Factor 3-selenium per day to a rat prevents death from liver necrosis.

The results suggest that hitherto unidentified and unsuspected trace elements may be essential for the well-being of mammalians, including the human. Nutrition research, having passed through a phase of vitamin discoveries, has now entered a phase in which new unsuspected trace element requirements become apparent. These agents are effective in quantities so small that water may play an important role as their dietary source 2/.

Selenium Derivatives

Selenium is in its properties quite similar to sulfur, but it is a relatively rare element. Its natural abundance in the surface crust of the earth is estimated to be 1 part in 10,000. In analogy to the numerous organic compounds containing sulfur, we

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2/ A further, more recent observation in this context showed that chromium, as a trivalent chromium complex, is apparently necessary for normal glucose utilization in the rat. It was identified as the active agent of the glucose tolerance factor. (K. Schwarz and W. Mertz, Arch. Biochem. Biophysics 85, 292 (1959)) know a rather complete series of selenium derivatives. Selenc[illegible] acids are analogous to sulfonic acids, seleninic acids to sulfinic acids, diselenides to disulfides, and selenium analogues of the sulfur containing amino acids, for instance, selenocystine, selenocystathionine, and selenomethionine, have been synthesized or isolated from seleniferous plants.

Almost 200 organic selenium compounds have been assayed for biopotency against liver necrosis in our test. Some typical examples are presented in Table 4. The ED_{50} (50% effective dose level against dietary liver necrosis) has been established for each of these compounds by tests at various concentrations in series of diets, using groups of 10 or more rats per dose. It was found that there are vast differences in efficiency between different forms (11).

With respect to biopotency in the rat, three main categories of selenium compounds must be distinguished: (a) many are more or less ineffective, even when given in large doses. The organism is unable to utilize them as source materials for Factor 3-active selenium; (b) another large proportion of selenium compounds shows an ED_{50} like that of selenite-selenium, i.e., 2 - 3 µg Se/100 g of diet afford protection. To this category belong most inorganic selenium compounds, but also the selenium-amino acids mentioned above, and many other organic selenium derivatives; (c) a few substances are more potent than selenite. Factor 3 from pork kidney powder (ED_{50} · 7 µg % Se) belongs here.

Synthetic organoselenium compounds with Factor 3-like potency have been obtained recently. During our fractionation studies, certain concepts had evolved of the physical and chemical properties of Factor 3 from natural sources. The active principle was an organic substance of low molecular weight, aliphatic in nature, with acidic properties, and containing selenium most likely in the oxidation level of selenite. By screening synthetic selenium compounds with these properties, and by stepwise synthesis of "tailor made" substances, synthetic compounds have been developed which are more potent than selenite and approach the biopotency of Factor 3. None of them, however, surpasses the activity of the latter.

Physiological Utilization of Trace Elements

We can draw two generalizations from these results. Both must be given serious consideration in any attempt to deal satisfactorily with problems of physiological trace element utilization:

(1) *Trace elements occur in different forms, and only certain forms are effective, while others are biologically inert*. This axiom of trace element supply and action has been much neglected in the past. Microanalytical determination of selenium, for example, does not give an indication of biopotency. From a comparison of the biopotency of natural source materials, on one hand, with their *selenium contents*, on the other hand, it is quite evident that not all the selenium in biological materials is present in the form of Factor 3. It is, therefore, a mistake to talk simply about "selenium" and its biological effects. Factor 3 potency can only be determined by bioassay.

(2) *To be biologically effective, trace elements must be present at their site of action within the organism in special, characteristic forms or compounds*. For individual trace elements the organism has highly specific mechanisms of absorption, transport, storage, entry through the cell membrane, enzymatic metabolism, and also excretion. Some of these aspects are documented quite well for certain trace elements, such as iron, copper, and manganese.

Methods of Selenium Determination

The amounts of selenium in nutrients and possibly water which are effective as Factor 3 are so small that it is not feasible to determine them accurately by means of the classical methods of selenium analysis. This is not a question of sensitivity as such. The procedures available for selenium determination are sensitive enough, but difficulties arise in the ashing or the digestion of the materials to be analyzed. Many selenium compounds are volatile. When only a few micrograms of selenium are present per gram of dry organic matter, in association with relatively large amounts of salts which are ubiquitous in specimens of biological origin, losses of selenium seem to be inevitable during wet or dry ashing. In many cases the element disappears altogether.

We have found that radioactivation analysis (12) is the method of choice. It ought to be pointed out that this method seems to have a great future in studies on biological trace element distribution; it is exceedingly sensitive and avoids the difficulties encountered in classical procedures.

In analyzing for selenium, the sample in its crude form is irradiated in a nuclear reactor or by another source of neutrons. Through an N ----> γ -reaction, one of the natural isotopes of selenium is converted into radioactive Se^{75}, which has a half life of ca 128 days and is easily handled. Subsequently, a carrier amount of unlabeled selenium is added in large excess (20 mg) before digestion of the material, and care is taken that wet ashing is thorough and complete. Following distillation with bromide, selenium is isolated in elementary form and counted for radioactivity. The losses which occur would be critical when only very small quantities of selenium were present. Due to the carrier-selenium they become irrelevant.

In general it must be stated that our analytical methods for trace elements are inadequate in many cases. Much is left to be done if we want to satisfy even the currently existing needs in trace element physiology and patho-physiology. Each element poses its own array of problems of determination, and new dimensions of sensitivity may have to be worked out for some in order to come to satisfactory methods of assessment.

As analytical methods which may be helpful in the future, x-ray emission spectrography (13), radioactivation analysis coupled with γ-spectrometry, and electron spin and nuclear magnetic resonance (14) may be mentioned summarily. However, as stated before, knowledge of the total amount of a trace element in a sample does not indicate its biological value; it presents merely an indicator of that biopotency which is maximally possible if all of the element were in the active, utilizable form. Hence, one would require a differential analysis of all the possible forms of an element before an analytical result could constitute an estimate of the biological value of a sample.

Such a differential determination of forms is out of the question if a highly reactive element, for instance, a transition element which readily changes its complexing ligands, is the object of analysis. For successful work on a trace element nutritionally essential, a reliable bioassay is indespensable, at best in combination with sensitive analytical means of determination. The biological assay seems to be here to stay. A potentially very fruitful, further approach may be the use of microorganisms for trace-element assay, but at the moment we do not yet have media pure enough for such tests.

Related Dietary Factors

With respect to the biological aspects of our example, selenium, some other important points should be brought out: In many deficiencies, including dietary necrotic liver degeneration, other dietary factors and the over-all dietary situation of the animal, exert a pronounced effect. In many of its documentations, selenium deficiency shows a particular association with vitamin E deficiency. As a matter of fact, a number of syndromes previously related to vitamin E deficiency are really Factor 3 selenium deficiency symptoms or are caused by the simultaneous lack of the two dietary agents. The latter is true for dietary necrotic liver degeneration.

For the production of this disease, both vitamin E and Factor 3 must be deficient simultaneously. Either one of the two, individually, prevents the fatal breakdown of liver function. However, the two agents do not simply substitute for each other. They are two independent dietary factors with different, but related sites and modes of action in intermediary metabolism. If only one of them is missing, chronic deficiency symptoms quite characteristic for this particular agent are seen.

The symptoms of vitamin E deficiency, such as sterility, central nervous system degeneration, and muscular dystrophy have been investigated thoroughly and are amply described in the literature. The symptoms of uncomplicated Factor 3 deficiency are now under study. They are distinctly different from vitamin E deficiency and involve pancreatic dystrophy, adrenal damage, muscular dystrophy, etc. Only if vitamin E deficiency and deficiency of Factor 3 occur simultaneously do we see the very acute, fatal necrotic degeneration of tissues 3/. In the case of the rat, the damage is primarily located in the liver, but in other animals other organs are primarily involved.

Concepts of Intermediary Metabolism

For the category of deficiency diseases requiring absence of two independent dietary factors before they develop, the term "ambogenous" has been proposed (15). An explanation for the etiology of ambogenous diseases is found in certain concepts of intermediary metabolism. It would seem that the two different agents are catalytically active in alternate pathways of metabolism. Both tocopherol as well as selenium readily participate in oxidation-reduction reactions, and the metabolic lesion found in the pre-necrotic, latent phase of dietary liver necrosis primarily involves

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3/ With the exception of muscular dystrophy, which belongs indubitably to the "necrotic" degenerative lesions but can be caused either by vitamin E or by Factor 3 deficiency alone.

oxidation. Therefore, it is conceivable that alternate pathways of oxidative metabolism, for instance, two parallel chains in electron transfer, may be involved and must be simultaneously deficient before necrotic degeneration, i.e., complete breakdown of vital functions of an area of tissue, occurs (4).

Originally, it was believed that cystine plays a primary part in the protection against dietary liver necrosis. Indeed, it was the first substance found to have a preventive effect, but more recent results have cast doubt on its role. L-cystine is commercially prepared from horn, hair, hooves, etc. It is ordinarily contaminated with small, but biologically significant quantities of Factor 3-active selenium (16). Relatively large amounts of such commercial samples of L-cystine are required in the diet.

The effective levels of the various factors preventing liver necrosis are shown in Table 5. Factor 3-selenium is by far the most potent. Approximately 560,000 parts of L-cystine were required to afford the degree of protection given by 1 part of Factor 3 selenium in the diet. The sample of L-cystine used for these experiments was found to contain 1.8 µg of the element per g weight. This can account to a large extent for the biopotency observed.

Effect of Sulfur-Amino Acids

However, more detailed additional studies of the interaction between vitamin E, Factor 3-selenium, and sulfur-amino acids have shown that sulfur-amino acids, per se, have a vitamin E sparing effect when added to the Torula yeast diet (17). For such studies, selenium-free methionine, and also selenium-free L-cystine prepared from commercial L-cystine, were used. Under our standardized conditions, the vitamin E sparing effect results in a delay of the development of liver necrosis, of approximately ten days. If the sulfur-amino acids are amply supplied the animals apparently can manage more effectively with the stores of vitamin E on hand at weaning. The exact mechanism of this sparing effect is not known, but recent studies indicate that there is an intimate interrelationship between vitamin E and sulfhydryl groups in the enzyme systems of the cell (18). This may permit a logical interpretation of the dietary results.

These interrelationships exemplify the fact that the requirements for any trace factor is dependent on other dietary variables.

It may vary greatly with the basic composition of a regime and may be dependent on the simultaneous presence or absence of other trace elements at the same time [4]. It goes without saying that other physiological aspects of the situation also have a pronounced determining effect on dietary requirements: Climate, work, conditions of stress, diseases and other vectors influence the nutritional needs of an organism profoundly.

At present we know at least 10 different species in which fatal pathological deficiency syndromes can be prevented by the normal, small amounts of Factor 3-selenium which are found in foods and feeds (Table 6). There is a wide spectrum of pathological changes which are seen in different animals. While liver necrosis dominates the picture in the rat, the mouse reacts to the same dietary conditions with a primary degeneration of the heart muscle, accompanied by peripheral muscle degeneration, liver necrosis, kidney necrosis, and pancreatic dystrophy. In birds, such as chicks and turkeys, simultaneous lack of vitamin E and Factor 3-selenium produces "exudative diathesis." This involves subcutaneous edema, changes of the serum protein levels, and also lack of growth (19, 20). The disease is quite acute and fatal. In different species, lack of a specific trace factor can produce very different deficiency diseases.

Extent of Selenium Deficiencies

Shortly after the discovery of selenium as the integral, active ingredient of Factor 3 it became evident that enzootic selenium deficiencies occur in many parts of the world. These deficiencies, known especially in sheep and in cattle as white muscle disease, stiff lamb disease, stillbirth, illthrift, etc., have had much commercial impact in the northwestern United States, New Zealand, Australia, Scotland, and other areas. The diseases are most pronounced in young animals. Severe muscle dystrophy, subendocardial degeneration, and calcification of the heart muscle are the most obvious changes. Their etiology has been a puzzle until recently.

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[4] An outstanding example is found in the well-known interrelationship between copper and molybdenum. See R. F. Miller and R. W. Engel, in "Symposium on Interaction of Mineral Elements in Nutrition and Metabolism," Federation Proceedings 19, 666 (1960). Molybdenum toxicity resembles copper deficiency and can be overcome by supplemental copper. Hypercuprosis, vice versa, can be treated by molybdenum.

Addition of 1/10 of a part per million of selenite-selenium to the feed of these animals, or the single application of a suitable amount of selenium to their mother, will prevent the fatal defects (21, 22). Today selenium compounds, mainly selenites, are used with great success in vast numbers of animals to protect against these lesions. The levels of selenium are physiological and the application is kept under veterinary control. In future studies improvements may come from the replacement of inorganic selenium salts by organoselenium compounds of high Factor 3 potency.

It can be expected that organoselenium compounds are less toxic than selenite or selenate. However, even for the latter substances the therapeutic index, i.e., the spread which exists between the dose level effective in the prevention of deficiency diseases, on one hand, and the toxic dose level, on the other hand, is satisfactory and normal. The minimum, chronic toxic dose for selenite is in the vicinity of 300 to 400 μg% in the diet. The spread, therefore, is roughly of the order of 1 to 100. It is better than that for most other dietary agents, including the known amino acids.

In considerations of toxicity the same basic principles must be applied which have been outlined above for the biological potency in the prevention of deficiencies: Different compounds of one element must be discussed and treated individually. Not only potency, but also toxicity varies greatly for various compounds of an essential trace factor. By systematic analysis both of biopotency and toxicity, one is able to develop preparations best suited for practical application.

Selenium and Human Health

It is logical to assume that Factor 3-selenium, which appears to be essential for the well-being of so many different species, also plays a role in the preservation of human health. This remains to be clarified in carefully planned studies, partially under way at present. In the context of this monograph, and speaking from a medical point of view, we wish to point out that yet another, wider import must be attributed to trace elements in another area: Some of the impressive beneficial effects produced by mineral waters, as they have been used for thousands of years around the world in spas and resorts with mineral springs, may be related to essential trace elements contained therein.

TABLE 1

Composition of Basal Diet

Torula yeast 1/	30
Sucrose	59
Lard, Vitamin E-free 2/	5
Salts 3/	5
Vitamin powder 4/	1

1/ Lake State Yeast Corporation, Rinelander, Wisconsin

2/ Vitamin E-free animal fat, stripped by molecular distillation (Distillation Products Division, Rochester, N. Y.)

3/ $CaCO_3$, 543.0 g.; $MgCO_3$, 25.0g.; $MgSO_4$, 16.0 g.; NaCl, 69.0 g.; KCl, 112.0 g.; KH_2PO_4, 212.0 g.; $FePO_4 \cdot 4H_2O$; 20.5 g.; KI, 0.08 g.; $MnSO_4$. 0.35 g.; NaF, 1.0 g.; $Al_2(SO_4)_3 \cdot K_2SO_4$, 0.17 g.; $CuSO_4$, 0.9 g.

4/ Lactose, 88.68 g.; thiamine HCl, 40 mg.; riboflavin, 25 mg.; pridoxine HCl, 20 mg.; D-calcium panthothenate, 200 mg.; choline chloride, 10 g.; niacin, 1 g.; menadione (2-methyl-1, 4-naphthoquinone) 10 mg.; folic acid, 20 mg.; biotin, 10 mg.; and vitamin B_{12}, 1 mg. Vitamin A acetate (1 mg. per cent) and vitamin D (0.01 mg. per cent) were added to the diets dissolved in a small amount of ethanol (0.5 cc per 100 g. of diet).

TABLE 2

Course of Dietary Necrotic Liver Degeneration in the Rat

		Duration in days	
		Fischer strain	Sprague-Dawley strain
INITIAL PHASE			
Macrosc.	Normal	11	25-35
Microsc.	Normal		
Electron microsc.	Beginning changes in cytoplasm seen on 7th day		
Metabol.	Normal(?)		
LATENT PHASE			
Macrosc.	Normal	8	10-20
Microsc.	Beginning fine changes; degenerative microbodies		
Electron microsc.	Severe degeneration of cytoplasm and mitochondria		
Metabol.	Metabolic insufficiency, respiratory decline.		
TERMINAL PHASE			
Macrosc.	Acute necrosis	1-2	1-2
Microsc.	Massive changes		
Electron microsc.	----		
Metabol.	Death		

TABLE 3

Factor 3 Activity and Selenium Content in Purified Preparations from Pork Kidney Powder 1/

Prep No.	Units of Factor 3 2/	µg. Selenium found
4737	Inactive	< 1
4759	Inactive	<1
4760	72	7
4197 B	330	3.5
4757	410	9
4741	430	12

1/ From reference (10)

2/ Dietary rat assay against liver necrosis

TABLE 4

Relative Potencies of Selenium Compounds Against Dietary Necrotic Liver Degeneration 1/

	ED_{50} 2/ (μg. selenium in 100 g. diet)
Factor 3	.7
Sodium selenite	2.2
Selenocystine	2.4
Benzeneseleninic acid	6.7
Selenouracil	ca 60
Phenylselenide	134
Selenium (gray modification)	Inactive (> 300)
4-Carboxybenzeneseleninic acid	Inactive (> 300)

1/ Structures and other details see ref. (11).

2/ Effective dose level in μg. per 100 g. diet required for 50% protection

TABLE 5

Effective Levels of Factors Preventing Liver Necrosis

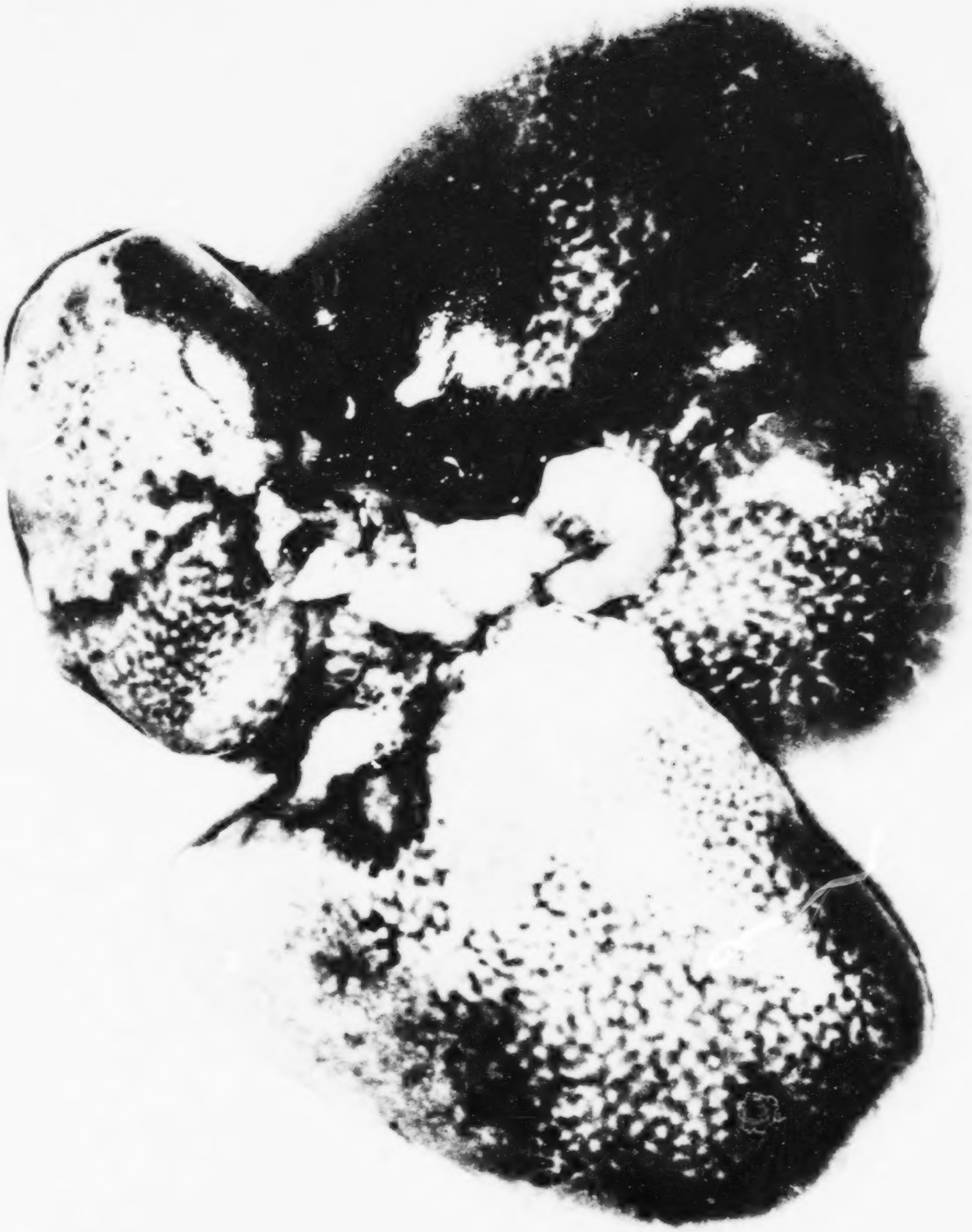
	ED_{50} (µg.%)	Ratio (F3 Se=1)
Selenium (as Factor 3)	.72	1
Selenium (as Se-cystine)	2.4	3.3
Vitamin E	ca 800	ca 1000
L-cystine	400,000 (.4% in diet)	560,000

TABLE 6

Factor 3-Responsive Deficiency Symptoms in Different Species

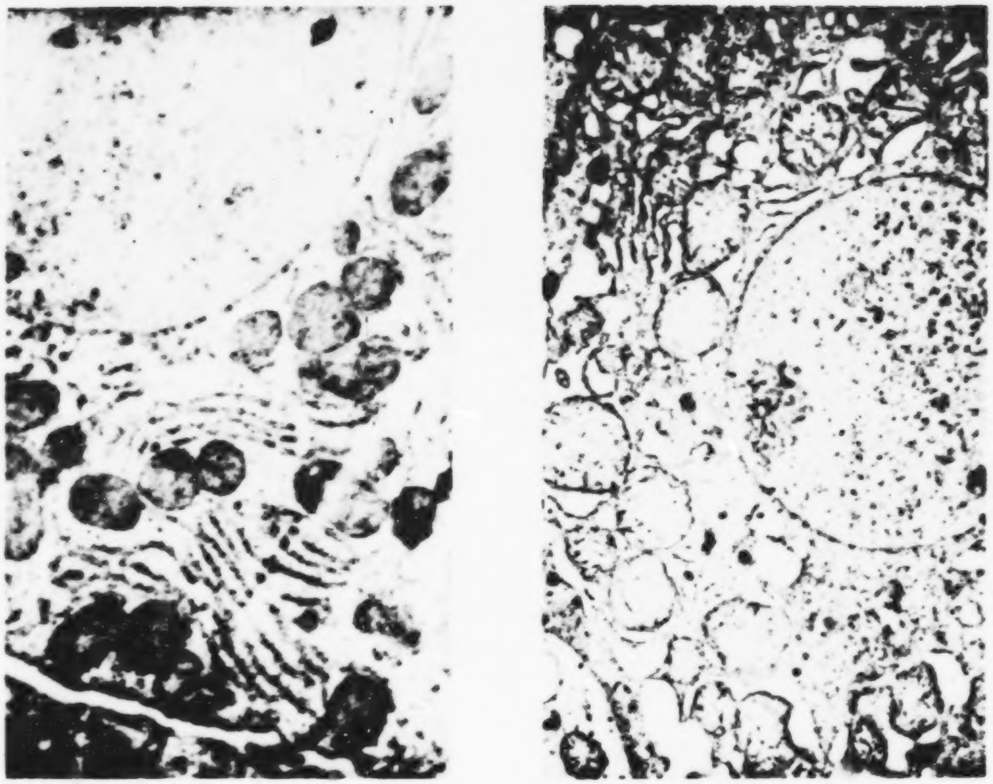
	Necrosis			Muscular Dystrophy	Calcifi-cations	Lung Hemorrhage	Pancreas Atrophy	Serum Protein changes	Exudative Diathesis
	Liver	Kidney	Heart						
Rat	+	+	(-)	(+)	+	(+)	(+)	+	---
Mouse	+	+	+	+		(-)	+	+	---
Rabbit	+					+			
Mink			+	+					
Hog	+			+					
Sheep			+	+	+				
Calf			+	+	+				
Horse				+					
Chicken				(+)				+	+
Turkey				(+)				+	+
Trout	+								

+ Pronounced pathological changes
(+) Not always detectable
(-) Occasionally found
- No pathological changes
Open spaces: Not yet investigated



Schwarz: Fig. 1

Dietary necrotic liver degeneration in the rat (From ref. 3)



Schwarz: Fig. 2

Electron micrographs of rat liver.
Left: Normal control. Right: After seven days of dietary treatment. Note swelling and rounding of mitochondria, conversion of intermitochondrial, elongated profiles into cysts (Courtesy of Dr. M. G. Piccardo).

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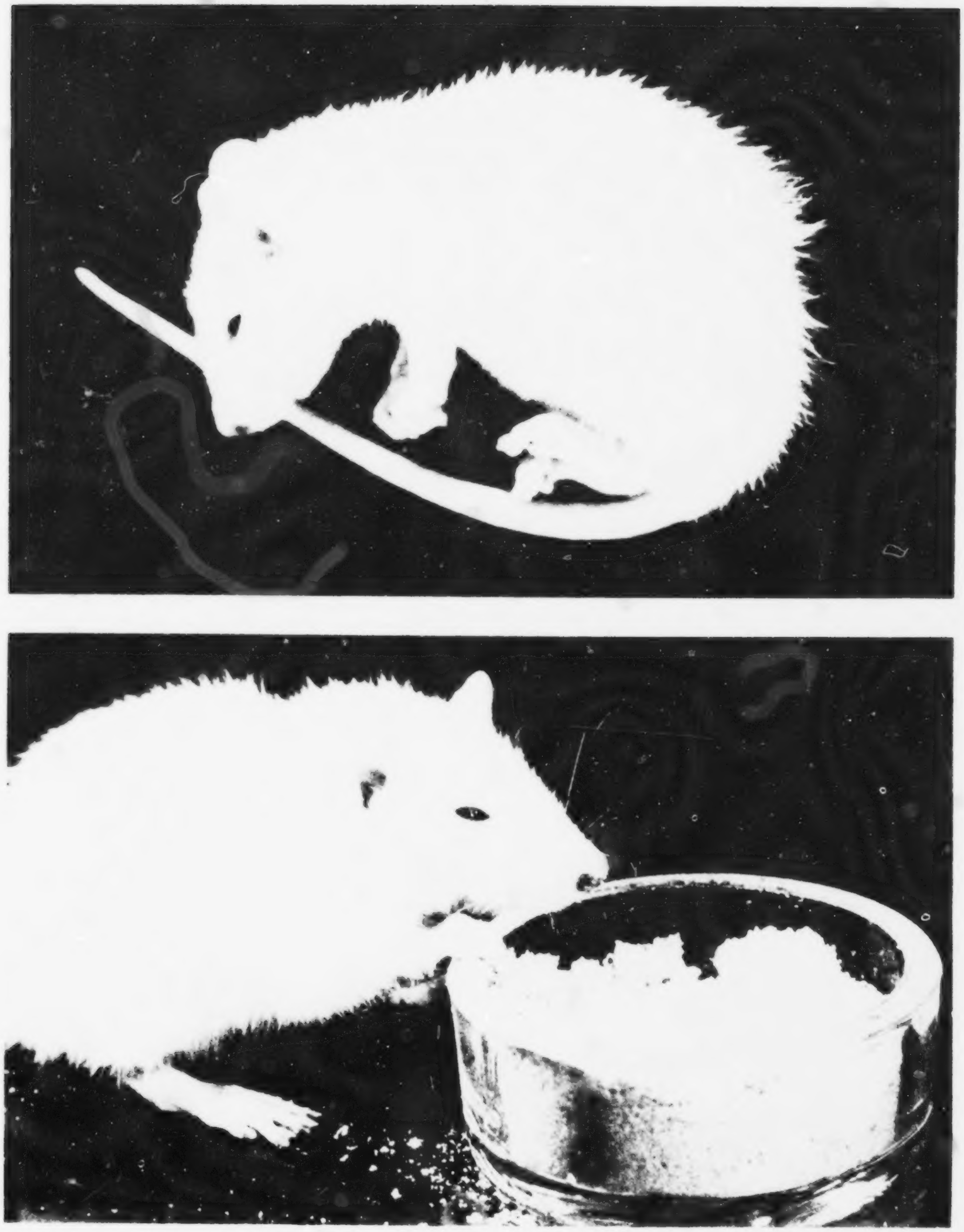

Schwarz: Fig. 3

Top: Rat dying from dietary necrotic liver degeneration.
Bottom: Same animal 15 minutes after intrajugular injection of 125 mg. of glucose per 100 g. of weight (From ref. 7).

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EFFECTS OF TRACE AMOUNTS OF VANADIUM

By

Dr. H. E. Stokinger

The purpose of this presentation is to summarize existing information on the physiologic effects of trace amounts of vanadium in the animal and human body. Interestingly enough much of that information which I am to present was stimulated by our search for early sensitive indicators of response to vanadium among industrial workers, as part of our research program in industrial toxicology.

I will describe several biochemical transformations, thus far known, that are moderated by trace amounts of vanadium. These are: (a) the interference with cholesterol and phospholipid synthesis, (b) interference with sulfur amino acid metabolism, (c) its stimulating activity for monoamine oxidase, (d) its inhibitory action on liver succinic dehydrogenase, and (e) its antagonism for iron. I am obliged to confess at the outset that I can describe only the effects of vanadium on the respective systems involved, for the intimate chemical mechanism by which vanadium produces these effects is not precisely known. Whether most of these activities occur because vanadium acts on a specific chemical grouping common to all is beyond our present knowledge.

Cholesterol Synthesis and Atherosclerosis

The possibility of vanadium having a metabolic function is not new. As early as 1899, French workers Lyonnet, et al. (1) showed that small doses of vanadium compounds resulted in increased growth rate of guinea pigs. Similar observations of alteration of metabolism were made by Jackson (2) and Martin (3). I will now present recent, unmistakable evidence for a metabolic role of vanadium; namely, that related to cholesterol synthesis. First, the evidence obtained from animals, then that from man.

Mountain, Stockell and Stokinger (4) in 1955 called attention to the capacity of orally administered vanadium to lower free cholesterol in the liver of rabbits and to restrict significantly elevation of free and total plasma cholesterol in rabbits on a 1 per cent cholesterol diet. Moreover, the important additional observation was made that the vanadium-fed animals invariably had less extensive areas of aortal placques, than those receiving cholesterol alone. Important also was the finding of no intracellular lipid deposition in the renal tubules in the vanadium-fed rabbits, whereas lipid deposition occurred in 50 per cent of the animals not fed vanadium. Of greatest interest in the present discussion of the effects of trace amounts of elements, is that these changes occurred when levels of vanadium in the liver were less than 0.5µgV/g tissue.

Evidence For the Role of Vanadium

Concurrently and independently, Curran (5) showed that vanadium added to rat liver cell clusters, decreased the incorporation of C^{14}-carboxy-labeled acetate into cholesterol. Vanadium was more effective than Fe in this respect. Effective vanadium concentrations were 5×10^{-5}M. Consistent with the findings of Mountain, et al., was the subsequent demonstration of Curran and Costello (6) that nontoxic dietary levels (0.05%) of $VOSO_4.2H_2O$ reduced excess aortic cholesterol in cholesterol-prefed rabbits by inhibition of endogenous synthesis of cholesterol. The finding that provided the most incisive and unequivocal evidence for the role of vanadium in cholesterol metabolism was that of Curran and associates (7) who reported vanadium to inhibit cholesterol biosynthesis at two steps, the conversion of hydroxymethyl glutarate to methyl crotonic acid and the conversion of mevalonic acid to cholesterol. Effective vanadium concentrations were 2×10^{-4}M. So much for the evidence from studies in vitro and in animals.

In man, vanadium performs similar effects on cholesterol metabolism. In trace amounts, vanadium inhibits the biosynthesis of cholesterol in human central nervous system tumors as shown by reduced incorporation of C^{14}-labeled acetate into cholesterol (8). Vanadium lowers serum cholesterol levels in normocholesteremic men by 20 per cent (9). Moreover, among vanadium mill workers, the level of serum cholesterol showed significant reduction (205 mg% vs 228 mg% among control workers) according to Lewis (10). It should be noted that these effects occur at extremely low levels of vanadium in the body; inhibition of human tumor cholesterol synthesis occurred at from 1.2-1.5µgV/g dry tumor; the 20 per cent reduction of serum cholesterol levels occurred when urinary excretion of vanadium averaged but 46µg/l at the time when reduced serum cholesterol levels were noted.

The Status of Epidemiological Evidence

The last link important to the present discussion is epidemiologic evidence that might support the contention that environmental vanadium (water, milk and food sources) may have health significance. Enterline and Stewart (20) have carefully mapped the geographic areas of the U. S. for coronary heart disease for white males and females. It was striking that in those age groups in which coronary heart disease is an important cause of death, the age-adjusted death rates were found to be roughly twice as high in some states than in others. Moreover, the geographic differences do not seem to be due to differences in urbanization and they appear to affect males and females alike.

Unfortunately the closing link in the chain of evidence, the association of known contents of vanadium in water, milk and food in the various areas of high and low mortality from heart disease, is almost nonexistent. A beginning has been made in Wyoming by Tank and Storvick (23); the vanadium content of water has been determined in 15 geographic areas of that State. Wyoming ranks 9th lowest among the States in incidence of coronary heart disease (20). Small amounts (20-150 ppb) of vanadium have been found in the waters of certain areas of New Mexico which ranks lowest of all the States in deaths from coronary heart disease (21). Vanadium was not found in those waters of New York State that have been analyzed; this State is that having the highest incidence.

Need For Basic Analytical Data

Thus the future work area in epidemiologic study of the effect of vanadium lies in obtaining a vast amount of precise analytic evidence of its concentrations in water, milk and food in those sections of the country showing extremes of mortality from coronary heart disease. Incidentally, similar evidence is needed to substantiate the epidemiologic report of Gentry (22) relating the presence of beryllium in the soil to incidence of sarcoidosis.

This common analytic need suggests therefore, a broad, concerted analytic attack throughout the U. S. for the presence of certain trace metals on which there is highly suggestive epidemiologic evidence of disease connection. Because of the vastness of the effort, precise areas for each element must be carefully mapped according to the epidemiologic evidence.

Effect of Vanadium on Phospholipid Metabolism

Vanadium in trace amounts also increases the oxidation of fatty acids of phospholipids of the liver in vitro (11). Not only does vanadium affect catabolism of phospholipids, but recent evidence from Synder and Cornatzer (12) suggests that vanadium inhibits the synthesis of phospholipids as well. Animals injected with vanadyl sulfate in a dose of 3.75mgV/kg responded with significantly reduced incorporation of P^{32} into phospholipid.

Effect of Vanadium on Sulfur Metabolism

Studies by Mountain, Delker and Stokinger (13) have shown that trace amounts of vanadium in the diet of animals results in lowered content of the sulfur amino acid, cystine, in the hair, indicating interference with sulfur metabolism. They later showed (14) that a similar reduction in fingernail cystine content of man resulted from exposures that occasioned no other clinical manifestation, and have suggested this as an early indicator of response to vanadium exposure. These changes occurred at urinary vanadium levels above µg/l.

New light has been thrown by Cornatzer (12) on the probable mechanism by which these cystine changes occur; a reduced sulfhydryl content of the acid soluble fraction of liver and an increased turnover rate of protein sulfur was found in animals treated with vanadium.

Effect of Vanadium on Enzymes

A specific inhibition of the sulfhydryl-dependent enzyme succinic dehydrogenase of liver, but not of kidneys of animals ingesting trace amounts of vanadium has been found by Mountain (15). This reduction in liver function has been interpreted as possibly accounting for the well-known increased body weight of animals fed small amounts of vanadium. Through what enzyme group(s) vanadium acts to effect this inhibition is unknown.

Vanadium has also been shown to be the most potent of the transition elements in catalyzing the oxidation of pressor amines derived from decarboxylation of amino acids (16); 10^{-5}M vanadium increased oxidation 13 per cent and 10^{-1}M, 125 per cent in vitro. Similarly vanadium was found the most potent of 18 metals in catalyzing the oxidation of the pharmacologically important catechol amines, 5-hydroxy tryptamine (serotonin), adrenalin, noradrenalin and others (17). Pentavalent and tetravalent vanadium compounds were equally effective in these in vitro studies. Two µgV/ml of reaction of volume effected these oxidations.

The importance of these findings is twofold: (a) in suggesting the possibility that vanadium might be the metal activator for monoamine oxidases, and (b) in possibly accounting for the intense vascular effects produced by vanadium on the spleen, kidney and intestine, and increased peristalsis allayed by adrenalin injection (18). Vanadium-treated dogs, however, responded with a *decreased* urinary excretion of the oxidized catechol amine 5-hydroxy tryptamine (19); no evidence was found for an increased catalysis of oxidation of this amine at any vanadium dose tested.

Capacity of Vanadium to Mobilize Iron

Lastly, and seemingly less striking from the standpoint of body metabolism is the capacity of vanadium to mobilize Fe in the animal body (15); Fe was moved *into* the liver and spleen by vanadium fed at levels of 100 and 150 ppm vanadium as V_2O_5, and out of the liver and spleen at dietary levels of 250, 500 and 1000 ppm vanadium. Changes in tissue Fe content caused by vanadium were more pronounced in the liver than in the spleen, and amounted to a maximum of +264 per cent at the 150 ppm vanadium level vs +145 per cent for the spleen; maximal Fe losses from the liver were -72 per cent compared with -26 per cent for the spleen at the 1000 ppm vanadium level.

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EFFECTS OF TRACE AMOUNTS OF ZINC

By

W. E. C. Wacker

The biological functions of zinc have been determined through the study of its association with specific proteins having enzymatic activity.

The discovery in 1940 by Keillin and Mann that carbonic anhydrase is a zinc metalloprotein and the recognition over the past six years by Vallee and his co-workers that the DPN dependent metallohydrogenases, carboxypeptidase and alkaline phosphotase are also zinc proteins has defined an indispensable functional role for this element in a number of important metabolic reactions. These findings, in isolated systems, account for the previously known presence of zinc in virtually all living organisms and serve to explicate, in functional terms, the biological effects of zinc deficiency in plants and animals.

The definition of the precise biological role of metals can only be approached through the study of such isolated functional systems. Measurements of the concentration of metals in body fluids or tissues cannot substitute for the integrated assessment of composition and function which is possible in isolated purified systems. In fact, tissue content may be grossly misleading in terms of functional importance as in the case of cobalt. Although its concentration in tissue is vanishingly small, yet as a component of vitamin B_{12} it is essential to life.

Conversely the presence of metals whose biological function is currently unknown does not allow the presumption that their occurrence represents a toxic accumulation. The element cadmium serves as an example of this type although chemically similar to zinc it does not have any known functions and its presence in tissue has been attributed to toxic exposure. The recent isolation of a specific protein from horse kidney containing 3 per cent of cadmium however implies a functional role for this element and renders unlikely speculations concerning its toxicity which were based solely on tissue analysis.

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OPEN DISCUSSION

DR. WHITTENBERGER: The papers are now open for discussion. I hope each participant will feel welcome to ask questions or make comments, even though his own field is not that of the speakers.

DR. WEISS: I have two questions directed to Dr. Stokinger. The first is: What natural foods contain a significant quantity of vanadium, foods in which human ingestion might play an important role?

The second is: Do you care to comment on the unusual situation in which ascideans accumulate vanadium, and particularly, with reference to the vanadium role in their biochemistry?

DR. STOKINGER: I have been unable to find any authentic analyses of the amount of vanadium in food. The chief sources of human exposure to vanadium are unknown to me (outside of minute traces present in some waters and a few plants), except in the specific exposures of industrial workers. The latter are the oil workers, who tend oil-fired burners from which residual ash contains up to 50 per cent of pure vanadium pentoxide. Exposures occur, of course, among the people who work in vanadium mines and mills. But I have been unable to identify any food sources of vanadium.

DR. WEISS: Is this because analyses of many substances have not been made for vanadium?

DR. STOKINGER: I think possibly it is.

DR. WEISS: Are there any plants which may accumulate vanadium, as tea accumulates fluoride?

DR. STOKINGER: There may be, but to my knowledge this never has been reported. I haven't explored all possible sources of information, but it certainly isn't reported in any readily available literature, let's put it that way.

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In regard to vanadium in tunicates, in ascidians; vanadium is not functional, as I understand it, the same way that iron is as a respiratory pigment in hemoglobin or magnesium chlorophyll in plants. Vanadium exists as a chromogen in a specialized cell whose function is unknown.

DR. WHITTENBERGER: Mr. Durum, do you have anything to add about the occurrence of vanadium?

MR. DURUM: Not in addition to what was discussed this afternoon. Vanadium does occur in very low concentration in many of the river waters examined thus far.

DR. WHITTENBERGER: I would like to ask Dr. Wacker about the occurrence of vanadium in tissue.

DR. WACKER: It is extremely rare to find vanadium in most biological materials. In fact, we use vanadium as an internal standard in the spectrographic method we employ for routine analyses.

DR. WHITTENBERGER: Other questions, comments?

DR. GREENWOOD: I would like to inquire about the sensitivity of the spectrographic procedure for the determination of the different trace elements. About what are the limits of sensitivity of it? Does the sensitivity vary for the different elements?

MR. DURUM: Sensitivity will vary, of course. Some elements are extremely sensitive spectrographically; to as little as 0.0001 per cent of dissolved-solids concentration in water. Copper is one element in that category. Others, such as zinc and arsenic, are less sensitive - perhaps in the range 0.1 per cent of dissolved solids. Most elements are detectable to about 0.0001 per cent of dissolved solids. Thus, there are some limitations in quantitative measurement of a specific element. Overall, however, accuracy is in the range of about plus or minus 15 per cent of the actual concentrations.

DR. MERTZ: I would like to inquire about the spectrographic analysis. What are its limits of sensitivity for the determination of chromium?

MR. DURUM: Chromium has two analytical lines, at wave lengths 4254 Å and 3021 Å. The first has a concentration range .0003 to 1 per cent of the concentration of dissolved solids, and the second .001 to 1 per cent. Thus we are talking about sensitivity to 1 microgram or less.

The spectrographic analysis of the residue is made with a standard matrix which is based on the average composition of the water of North America as determined by Clark in 1924. The standard samples are arced on the same plate as the unknown samples. It is not necessary to ash the residue.

DR. WHITTENBERGER: Dr. Wacker, would you like to add anything about the use of spectrographic methods as applied to tissues?

DR. WACKER: We use a spark method, the porous cup method of Feldman, which I think is better adapted for biological work. In this technique the sample is ashed and then dissolved in 6N HCl prior to analysis. As for chromium, the lower limit of sensitivity with this method is about one-tenth of a microgram per gram of original material.

In addition to the spark method using photographic recording, we also have a direct reading flame method, using a high energy cyanogen oxygen flame and electronic recording. This technique is not fully operational as yet. The sensitivity is about the same.

DR. WHITTENBERGER: We have heard quite a number of reports from biochemists and toxicologists this afternoon, with occasional reference to epidemiologic investigation. Dr. Anderson, do you have any comments to make about any of the points that have come up today?

DR. ANDERSON: Yes. Referring to statements about arteriosclerosis, I think we must be extremely cautious in drawing any conclusions. A reference was made to one or two published articles. They are very good articles and no criticism is warranted of them as they are prepared, but we are faced with many diagnostic difficulties. We must define clearly just what we are talking about. May I cite an example from outside of the field of arteriosclerosis.

When I was with the Massachusetts State Health Department we were very much interested in pneumonia, and everybody was quite excited when we found that down on Cape Cod we had an amazing number of lobar pneumonia deaths. Many suggested that this might be due to the humidity conditions out on the Cape. But when we reduced the conditions down to a simple factor, we found merely that the doctors on Cape Cod were calling all cases lobar pneumonia and none bronchopneumonia, so that the whole phenomenon was due to nothing but diagnostic differences.

With something as obscure as arteriosclerosis and coronary diesease, in fact this whole atherosclerotic syndrome, I think we must be extremely cautious with respect to our statistics. Our statistics themselves are worthwhile, and I am not suggesting that they should not be used. But when we see statistical variations from one part of the country to another, and recognize the difference in diagnostic terms - using different diagnostic criteria - it is obvious that we must be extremely cautious in attempting to draw conclusions.

Furthermore, we must remember that we are dealing with a problem of classification of causes of deaths. In reporting mortality statistics we have to "allow a person to die" from one thing only. The published statistics do not show those other conditions that were present in the individual, and which may have been very serious.

A study was reported in the literature during 1960 concerning the effect of certain mineral components of water supply as they might affect the amount of coronary disease and atherosclerosis. This study purported to show that there was an inverse relationship between the prevalance of these components and the hardness of the water. This report was commented upon editorially in the Journal of the American Medical Association, and - perhaps unfortunately - the story was published in various newspapers and news magazines. The stories implied that the purification of municipal water supplies, especially treatment to reduce the hardness of water, is adding to the danger of dying from coronary disease or atherosclerosis. An equally good correlation might be drawn, incidentially, between similar statistical data and the results of the Presidential election of 1956. It is a masterpiece of bad statistics even though it is supported by electronic computer calculations.

DR. WHITTENBERGER: Thank you very much, Dr. Anderson. Do others have contributions to this discussion?

DR. LACKEY: I have two questions addressed to Dr. Wacker. We have been talking this afternoon about metals in plants and in animals. I have no difficulty understanding the pickup of metals and other elements from soil and water by plants. In the case of animals, however, the metals discussed here are present in complex compounds, and we are talking about water quality. I would like to know how the metals with which you gentlemen have been working are picked up by animals from water. Are they picked up directly by going through a series of complex syntheses in the animal? Or are they picked up from a food chain and through a complex system of syntheses? That is the first question.

The second question is: Do you Dr. Wacker, regard the mere presence of a substance in a biological system as indicating it has a biological function? I will explain what I mean. One per cent of kelp, macrocystis, for example, by weight is sulphur. Those are the two questions.

DR. WACKER: If I understand correctly, you are asking in what manner the metals are taken up by animal tissues?

DR. LACKEY: How they get from water to the substance or organ in which they are found.

DR. WACKER: I see, yes

For many elements these aspects of the physiology have not been ascertained. Iron reactions are about the best worked out; this element is absorbed from the gut and it apparently is absorbed better in the ferrous state, although this point has been argued back and forth. Also, it is better absorbed in the presence of substances which dissolve iron and do not precipitate it. After it is absorbed it is picked up and carried by a specific protein present in serum which binds iron preferentially. It is transposed to the liver where it is deposited and stored in the form of insoluble protein called ferritin; the stored iron is in equilibrium with the circulating iron and that present in the various cells which require the metal. In this regard iron is a necessary component of the respiratory enzymes of all cells. However, the major function of iron is that which is present in hemoglobin where it serves as the site of oxygen building.

The second question was whether merely finding an element to be present in biological material indicates that is has a function. No, I don't believe that this would necessarily be true. However, the point I was making in regard to cadmium is that we have found cadmium to be associated with a specific biological substance - namely, with the protein metallothionein. This specific protein always has a constant metal content. This certainly implies that cadmium in this protein has a biological function, although it doesn't prove it absolutely.

DR. MORRIS: May I follow this discussion further? Is it possible that the protein-cadmium compound which you found, instead of serving a synthetic biological function, might simply be a detoxification compound; in the latter case the cadmium might then be related to toxic symptoms rather than to beneficial ones?

DR. WACKER: Yes, it is possible, but, I think there are objections to this hypothesis. At first glance such a role seems to be fairly reasonable, as the protein has a high sulphur content which would allow it to bind metals avidly, and in addition to cadmium it also contains zinc. Therefore, the protein could be formed in the kidney to bind extraneous metal. The main fact which argues against this hypothesis is the finding of a very constant amount of cadmium in a number of preparations that we have isolated. If the metal content were simply the result of "straining" cadmium out of the urine or blood, then one wouldn't expect to find the protein to contain a constant amount of the element. Furthermore the protein isn't saturated with cadmium; when isolated it still has free sulfhydryl groups which are capable of binding more metal.

DR. WHITTENBERGER: Dr. Monty, would you like to add any comments in answer to these questions?

DR. MONTY: I would like to add a general comment concerning the difficulty in establishing whether a trace element, or any nutritional constituent for that matter, is essential or not. An example concerning molybdenum may help to illustrate the sort of problem that can arise.

It has been possible to demonstrate that the addition of small amounts of molybdenum to certain experimental diets will stimulate the growth of chicks. The addition of inorganic sulfate to these diets appears to increase the requirement for molybdenum. However, armed with only these observations, we cannot logically distinguish between two possible explanations for the phenomenon. Does the chick have a requirement for molybdenum which is potentiated by dietary sulfate, or is the chick subject to a sulfate toxicity which can be alleviated by molybdenum? Until we know what molybdenum is doing mechanistically, we cannot resolve this problem.

Such problems involving an understanding of the mechanism of interrelation between two or more dietary factors are all too common in contemporary nutritional research endeavors.

DR. BYERRUM: Since we are talking about cadmium-protein, I have a question or two about it. I wonder, first of all, whether the protein in horse kidney contained all of the cadmium in the kidney and, secondly, whether you tried to isolate such a protein from any other animals.

The reason I ask the second question is that in our laboratory we tried to isolate a cadmium-containing protein from dog kidney after giving an animal a small amount of radioactive cadmium. The first thing that we noticed was that when we tried various separation techniques on the proteins of the kidney, cadmium was discovered to be spread more or less uniformly among a group of proteins. It is perhaps true that you can isolate, ultimately, a cadmium-containing protein from dog kidney, but this material would contain only a small portion of the total cadmium in the kidney.

DR. WACKER: At least 60 to 70 per cent of the cadmium in horse kidney is present on the protein. It is a very strange protein in certain respects -- it has a very small molecular weight (about 10,000), and is extremely soluble. It does not precipitate by the usual protein-precipitating procedures. As a matter of fact, we isolate it by precipitating everything else and then chromatographing the remaining supernatant material to determine the cadmium protein.

DR. McKEE: One trace element that is entering our water supply in increasing amounts is hexavalent chromium. I wish to ask Dr. Byerrum, who has recently been doing research on this substance, to tell us what he thinks about the limit of .05 parts per million (ppm) for this metal set by the Public Health Service drinking water standards. Could you give us a brief statement of your thinking on chromium?

DR. BYERRUM: I can report what we have found in a two-year study with rats in which various levels of chromate have been fed over this period. We find that with lower levels of chromate (0.1 - 5 ppm) there is little accumulation in any tissue that we studied.

DR. McKEE: You are referring to chromate concentration in the water.

DR. BYERRUM: In the water. When the concentration in the water is increased above 5 ppm there is some accumulation noted in tissue over the two year period. As a matter of fact, the higher the chromate level, the more accumulation results. The accumulation is principally in the liver and kidney.

However, examination for gross pathological changes or for microscopic pathological changes in a large group of tissue, including liver and kidney, has revealed none in any of those animals receiving up to 15 ppm of chromium in the drinking water. We do note some accumulation, but we don't find any pathological changes; nor is there any change in growth rates, or food or water consumption in any of the groups receiving up to 15 ppm of chromate in water.

DR. MONTY: Earlier today Dr. Byerrum and I were comparing notes on chromium ions as bacteriostatic agents. Chromate, in particular, is very effective indeed in inhibiting the growth of many microorganisms, probably because it is such a good oxidizing reagent. It occurred to us that here is something that might be advantageously added to water some day to aid in control of bacterial contamination. Our present knowledge would indicate that bacterial tolerance to chromate is several orders of magnitude lower than is the tolerance of higher animals.

DR. BYERRUM: In this regard, we made two studies -- one for a year and one for two years -- using a large group of control animals which received no added chromate, some 50 animals in the one year study, and 50 in the two year study. In addition, groups of 30 animals received various concentrations of chromate up to 15 ppm in the drinking water. The survivability, if that is a good word, or longevity of the animals receiving chromate was greater than the controls. At the end of two years, a significantly greater number of animals were alive in the experimental groups than were alive in the control group. Precisely what the reason for this is, I am not prepared to state, but that is an experimental fact.

DR. LACKEY: I think if this is the case, we better add the chromium to the drinking water and not to the sewage.

DR. WHITTENBERGER: Dr. Nelson, do you have any comments at this time?

DR. NELSON: I have one question, which perhaps Dr. Mertz can clarify. If there is a human selenium requirement, would you expect that normal diet through factor 3 would supply this, or would dietary selenium make a significant contribution to it? If so, under what circumstances would the dietary and selenium sources other than factor 3 be significant?

DR. MERTZ: At the moment we do not know whether there is a Factor e-selenium deficiency in the human. However, such diseases have been recognized to occur enzootically in animals. At present, at least ten different species are known to develop fatal diseases which are preventable by Factor 3-active selenium compounds. One is tempted, of course, to think that a factor with protective effects against serious deficiency diseases in so many species plays a role in man as well. If one dares to conclude or draw implications from animal diseases, one should look for beneficial effects of Factor 3-selenium in degenerative, necrotizing diseases of the liver, the kidney, the heart, the musculature, the pancreas, etc.

There are methods through which this can probably be established. A simple determination of selenium levels in blood or of selenium excretion in the urine, for instance, should give us an idea of the status of the organism with relation to selenium supply. One could make a selenium balance, just as one can make a nitrogen balance. The small amounts of selenium which are biologically necessary and effective are, in general, present in nutrients.

The determination of these minute quantities is ordinarily not possible by conventional chemical or colorimetric assay techniques, since they are not sensitive enough; however, radioactivation analysis has been used to great advantage for this purpose.

Factor 3-selenium seems to be bound to proteins; thus one may need to look for a selenium deficiency wherever one finds protein deficiency, i.e., specifically protein malnutrition (kwashkiorkor). The latter is a widely occurring, severe deficiency disease prevalent in underdeveloped tropical and sub-tropical countries, but sporadically also occurring in New York.

From our data on the Factor 3-selenium contents of nutrients which we have accumulated through the years it is apparent that a highly refined dietary such as that consumed in the U.S.A. today may have comparatively small amounts of Factor 3. Beef steak and cow's milk, for instance, are poor sources, and so is highly refined flour. There is, in other words, the possibility that in some of the degenerative diseases seen in the American population, Factor 3-selenium deficiency plays an etiological part.

It has been our philosophy not to start clinical studies until we have clarified the chemical structure of Factor 3 and found the optimal conditions for its application. Over the last three years we have developed synthetic, organoselenium compounds which are more potent than selenite-selenium, and which may be less toxic. Having thus laid the groundwork for clinical application, we hope to have an opportunity to establish the significance of Factor 3-selenium for the human before too long. Preliminary investigations using one of these selenium compounds are under way, in Jamaica studying babies having protein malnutrition.

DR. STOKINGER: I wish to comment on Dr. Monty's paper. He described the complexities of the molybdenum systems and their relation to numerous biologic transformations. He emphasized the idea that there is a great interplay of antagonisms and synergisms associated with the element molybdenum. If this were true of all biologically active metals, of course, it would present a pretty gloomy situation; because it is very difficult to unravel such a complexity of interrelation in the biochemical field.

But I want to also, because of the gloomy outlook, to mention that this situation probably does not apply to all elements that we have heard discussed here. I would like to discuss a mechanism indicating this may not be true for all elements. It is this: whether or not an antagonism exists between one metal and another depends upon the specificity of the metal toward the biological component with which it is acting. If there is a high degree of specificity, a low disassociation constant, there then will be a lesser interplay with other elements.

Therefore, we would expect certain elements (I think vanadium is one of these, and possibly arsenic is another) to go their way undisturbed and accomplish what they do in the body with very little interaction and interplay with other elements. I might just leave this thought with you, because it is a discouraging and frustrating thing to feel that all elements in the body are involved in such a complex interplay; that one substance at a higher concentration will displace the other. I think it is not necessarily true for all elements. I certainly think molybdenum is one which displaces, and zinc another, but I consider that not all elements do not fall into this category.

DR. WHITTENBERGER: We will give Dr. Monty first chance to answer this.

DR. MONTY: I am not discouraged by the apparent "gloominess" of our problems. I prefer the optimistic viewpoint that these phenomena involving molybdenum will ultimately prove considerably less complex than our present state of ignorance would suggest.

I would like to point out some generalities that have already emerged from our studies of molybdenum. First of all, we have seen that three ions, molybdate, selenate, and tungstate, have qualitatively identical effects on the metabolism of E. coli. Several other ions undoubtedly would have the same action upon the organism were it not for the fact that they have other sites of action in addition, and that these latter effects obscure their potential inhibitory role in sulfur metabolism.

Secondly, in the nutrition of higher animals, we have begun to recognize a considerable amount of overlap in the physiological and chemical symptoms resulting from toxic dietary levels of molybdate and selenate. It would appear that these two toxic agents have some sites of action in common, and other sites of action which are perhaps unique to themselves. I think that when we have learned all about molybdenum toxicity, we will have learned a great deal about selenium as well. I find this a rather encouraging notion.

There is a subtle aspect of dietary metal poisonings that I mentioned earlier and would like to discuss briefly here. I will discuss the problem as it pertains to molybdenum toxicity, but I am sure that the general considerations will apply to many, if not all, metal toxicities of dietary origin.

As I stated earlier, there is a voluntary food restriction that accompanies the ingestion of excessive amounts of molybdenum. Some preliminary experiments have indicated that the ingestion of molybdate elicits a prompt unpleasant sensation in the rat. The animals develop the ability to associate this physiological response with some sensory attribute of the molybdate-containing diet. We do not know precisely what they taste or smell; the factor is probably not molybdate per se, however, but rather a chemical product of the interaction of molybdate with some other constituent of the diet. The important fact is that the animals develop the ability to recognize the toxic diet, and thus protect themselves against its effects to a degree by voluntarily limiting their food intake to a level approaching the minimum compatible with survival.

Although I do not have the data to back up this notion, I suspect that ingestion of molybdate via the drinking water is potentially more hazardous to the animal than ingestion of the same ion via the solid part of his diet. The reason is that the sensory detection of molybdate tastes very much like a large number of other salts. Thus, the animal is deprived of the conditioned sensory mechanism which enables him to minimize his ingestion of molybdate when it is encountered in solid foods.

DR. WHITTENBERGER: Dr. Wacker, do you want to add any comment?

DR. WACKER: I would like to point out that there are other examples, and I am sure Dr. Stokinger is aware of these also, of complex interactions between metals leading to deficiencies. Zinc is an example. The only mammalian zinc deficiency that exists as a natural disease is parakeratosis of swine. This disease is due not only to low dietary zinc, but to high dietary intake of calcium. In fact, the disease is produced when pigs are overfed with calcium in order to hasten their growth rate. The extra calcium actually produces zinc deficiency. Magnesium deficiency can also be produced by feeding excess calcium.

DR. WHITTENBERGER: I would like to follow up Dr. Monty's remarks with a question. He mentioned the remarkable ability of rats to select diet. I wonder to what extent this is true in man?

DR. MONTY: It is very important to recognize that in our experiments, the rats have not selected diets, but have discriminated against dietary constituents. Secondly, this discrimination was a conditioned response, something they learned to do by trial and error. However, the literature contains a few experiments which indicate at least a limited ability of rats to select *for* a dietary essential.

Human beings are better equipped mentally than are rats to make the associations upon which conditioned discrimination is based. However, our sense of taste and smell is not so well developed, generally speaking, or at least not relied upon as heavily as it would be if we were rats. So in practice the rats may perform better in a discrimination experiment than does the human.

It is not uncommon to hear a statement to the effect that a human child, if he were offered a wide enough choice of edible materials and protected from adult prejudices, would successfully select a proper diet for himself. I do not believe that such claims have any real experimental foundation. We all recognize that humans generally do not die of voluntary starvation, lack of water, or lack of sodium chloride. I am not sure that our innate abilities to select our diet cover very much more than these three problems of selection.

DR. WHITTENBERGER: As a respiratory physiologists, I would like to refer to a comment that Dr. Kehoe made this morning. Many of you are interested in the water aspect and tend to think of water to the exclusion of other environmental factors. Some of you are interested in nutrition, and thus think more about food and less about water. I would like to mention that some elements may enter the body through the air as well as through the diet and water -- lead, for example. We must always keep in mind the equilibrium of metals in the body as influenced by what is in the air, as well as what is in the water and food.

I would like to thank the speakers and the audience for their contributions to this discussion.

PHYSIOLOGICAL EFFECTS OF INSECTICIDES

By

Wayland J. Hayes, Jr.

Pesticides promote health directly through the control of vector-borne diseases and indirectly through increased and improved agricultural production. However, the extensive use of any biologically active chemical implies a potential hazard which must be evaluated.

Production and Mode of Use

Production and use of these materials have increased greatly since World War II. In the United States alone, the annual production of synthetic organic pesticides, which were essentially unknown before the development of DDT, reached a level of 569,927,000 pounds in 1956. While production of these synthetic materials has increased from essentially zero in 1942, production of the older, largely inorganic, pesticides has shown only a partial decline. Although something less than 200 basic chemicals are really important as pesticides, the situation is complicated for the practicing physician by the fact that thousands of different pesticidal formulations are labelled and sold in this country. The situation is further complicated by the fact that pesticides are now applied in ways which were either unknown or uncommon a few years ago. An example is the extensive use of aircraft for spraying operations.

Pesticide Mortality and Morbidity

The death rate from poisoning caused by all pesticides in the United States is slightly less than 0.09 per hundred thousand population. Strangely enough, most of the fatal accidents in this country still involve the older pesticides. For example, in 1956, there were a total of 152 accidental deaths caused by all pesticides and of these, 104 were caused by compounds older than DDT, including 54 caused by arsenicals alone. Over half of the cases occur in children. Thus in 1956; 94 of the 152 cases were in children under 10 years of age and 78 were in children under 3.

Both the types of compounds involved and the predominance of cases in children indicate the possibility of real improvement if

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people can be educated to use these materials with even greater care. The safety record in some other countries is not so favorable, especially in connection with the newer compounds. It appears likely that improved labelling and improved regulation of use in those countries would help to remedy the situation.

Information on morbidity is far less precise than that on mortality. In one study in which only serious cases were counted, the ratio of fatal to non-fatal cases associated with poisons was 1:13. In another study in which every report of trouble was counted as a case, the ratio was 1:750. The ratio is commonly estimated as 1:100 and this comes as close to the truth as is possible at this time.

One gets the impression that the newer pesticides cause more non-fatal accidents than the older compounds cause. This is what one would expect from the amount of the various compounds used. However, the conclusion must be taken with reservation because reports of fatal poisoning by pesticides in the lay and technical press are chiefly concerned with the newer compounds and yet the official statistics show that in this country the majority of deaths are caused by the older compounds.

Environmental Exposure to Pesticides

Incidental or environmental exposure to pesticides has been a source of difficulty only rarely, as for example, the allergic reactions of a few people exposed to vaporized lindane. Excluding acute poisoning following massive exposure, any ill effects are to be expected in persons whose exposure is intensive and prolonged. A study of such people is also the best single source of information about the potential long term hazard to the general population in the use of any particular compound.

Nothing is now known to justify the suggestion from some sources that pesticides currently constitute a serious threat to human health. Nor is there evidence that pesticides now are a significant cause of blood dyscrasias and other diseases. However, it is the duty of the Public Health Service to keep alert to such possibilities and, in addition to our own planned studies, we welcome reports of cases or any other relevant information.

Methods of Toxicity Studies

There is now a considerable amount of information about the clinical effects of poisoning by a wide range of pesticides in man. At least 48 of these compounds are known to have produced human poisoning. There is some useful information on the treatment of poisoning by many of them. However, much remains to be learned about symptomatology and treatment, as well as about the prevention of poisoning.

By necessity, first estimates of the safety of a pesticide must be based on its toxicity in experimental animals. Such study may provide essentially all the information that is necessary in evaluating the probable results of acute exposure, such as that involved in accidentally eating the compound. However, additional studies must be made if the mode of action of the compound is to be understood and the danger of occupational poisoning is to be evaluated.

The study of dermal toxicity in animals is more useful than the study of oral toxicity in predicting occupational hazards. However, differences in the reactions of different species to the same compound are such that studies must eventually be made in man himself. Such studies, in man, have been made of a number of compounds including DDT, schradan, dimefox, parathion, Dipterex, malathion, tetraethyl pyrosphosphate, and dinitrophenol.

Absorption Through the Skin

It may be nearly as valuable to know the magnitude of exposure by different routes as to know the toxicity of a compound. It has been known for some time, on the basis of animal experiments, that many of the newer pesticides, except DDT, are absorbed easily through the skin, even when not in solution. This property has been associated correctly with cases of poisoning following massive skin exposure, but the importance of dermal absorption in the more ordinary cases of occupational illness frequently has been missed.

Actual chemical measurement of the exposure of applicators to several compounds used in agriculture or in vector control has shown that skin exposure is greater (sometimes very much greater) than respiratory exposure when liquid sprays are used. However, not all work situations have been explored and additional studies need to be made.

It has been necessary to develop new techniques for measuring the exposure to pesticides because the older methods used for measuring exposure in factories and mines proved inadequate. The newer methods are useful not only in evaluating different routes of exposure, but also may be used in studying the efficiency of protective clothing and devices.

One aspect of toxicology that has been neglected is the quantitative study of absorption, storage, and excretion as influenced by intensity and duration of dosage. I am not aware of a general review, much less a book, on this matter. For some reason, the biotransformation of foreign compounds in the body has attracted more study; and an excellent book is available on this subject.

Storage of Pesticides in the Human Body

People generally are unaware of the variety of compounds that commonly are stored in us; and therefore they are shocked to learn that DDT is stored in essentially everyone in the United States.

It is known that storage reaches a steady state at any given level of continued dosage and gradually disappears when dosage is discontinued. The ratio of concentrations of a compound in different tissues may vary, depending on the dosage schedule, state of nutrition, and perhaps other factors. In some species, at least, storage is different in the two sexes. It seems likely that the dynamics of storage is not fully known for a single compound.

It is certainly clear that compounds differ widely in regard to storage. The behavior of the "bone-seekers," such as radium, is so different from that of the water-soluble drugs that it is customary to use different mathematical treatments in presenting data on the two kinds of materials. There is some justification for this because there is evidence that radium interferes with its own excretion, while most materials do not.

The point is that much more attention needs to be given to storage in order that its significance in different instances may be evaluated more meaningfully and in order that a general theory of storage may be formulated, if possible.

Pesticides in Relation to Water Supplies

As background for a discussion of pesticides in water, it must be recalled that the toxicity of these compounds to fish is high but largely unrelated to the toxicity of the compounds to mammals. For example, considering insecticidally-active compounds and isomers, all of the chlorinated hydrocarbon insecticides are more poisonous to fish than are any of the organic phosphorous compounds.

On the contrary, organic phosphorous compounds cause several deaths from occupational poisoning each year, while occupational exposure to chlorinated hydrocarbon insecticides has caused only a few deaths in the last 15 years. Methoxychlor, which is far less poisonous to mammals than DDT, is essentially as toxic to fish as DDT. Rats may be maintained for more than a year without injury on water containing 0.2 ppm of dieldrin, a concentration that kills fish in a few hours.

It is a curious fact that fish, like mammals, may store high concentrations of DDD (and presumably other chlorinated hydrocarbon insecticides) absorbed in small repeated doses. Such storage is not accompanied by observed injury.

The organic phosphorous insecticides gradually hydrolyze in water to form harmless compounds. The chlorinated hydrocarbon insecticides are highly stable; but some of them, such as DDT, are bound by soil (especially soil with organic matter) and rendered inactive.

Pesticides may enter water by direct application or by runoff from agricultural lands or forests. Direct applications may be made intentionally for the control of mosquitoes, gnats, and other pests. Such applications, as well as accidental direct contaminations usually associated with forest treatment, have been the cause of fish kills. Much more rarely, the runoff from treated land has been sufficiently rapid to kill fish in small streams.

Chemical study has indicated that insecticides may be present in surface water in concentrations too small to produce observable injury in fish. In no known instance has the concentration of a pesticide in surface water reached a level of real public health significance. Because of the extreme sensitivity of fish, they may be used, with a wide margin of safety, for the continuous bioassay of drinking water.

Conclusion

Largely because of careful labelling, the human safety record of the newer pesticide compounds is good in some technically advanced countries. In countries where labelling is poor or where illiteracy or irresponsibility tend to vitiate labelling, hundreds of cases of human poisoning have occurred among small populations within a brief period. Because of the usefulness of economic poisons and their future potential in the control of vector-borne diseases and also in the control of a wide range of pests of field crops, livestock, forests, and stored products, it should be our aim to improve their safety in order that they may be fully exploited.

Reference

1. This paper is based in part on a more extensive review by the speaker titled "Pesticides in Relation to Public Health," published in Annual Review of Entomology, Vol. 5, pp. 379-404, 1960. Annual Reviews, Inc., Palo Alto, California.

PHYSIOLOGICAL EFFECTS OF ORGANIC PHOSPHATE PESTICIDES

By

O. Garth Fitzhugh

In the past more attention has been focused on the biological than on the chemical contamination of water. The problem of non-biological contamination of our water supply is becoming increasingly significant. Many substances which get into our water supply have not yet been adequately studied chemically or pharmacologically. It is imperative that these contaminant substances be identified so that their toxicology can be evaluated. In sufficient amounts they can be hazardous to health, and may be lethal on acute or chronic exposure. Among the contaminants in our water supplies are the pesticides. These compounds have been used to control disease and to eliminate pests in agriculture and in the home. Although these agents are necessary, their use presents hazards which must be considered.

Poisoning may result from an acute exposure of sufficient quantities or from chronic exposure to small amounts of these agents. The Federal Insecticide, Fungicide, and Rodenticide Act was established to register and properly label these chemicals. The Miller Pesticide Amendment to the Federal Food, Drug, and Cosmetic Act provides for the establishment of tolerance levels for residues on raw agricultural commodities, and the Food Additives Amendment requires tolerances for residues of toxic substances in processed commodities. Tolerances are established on the basis of animal feeding experiments and are usually levels which allow a wide margin of safety based on growth, survival, histopathology, and other indices.

Inhibition of Cholinesterase Enzyme

Among the most widely used pesticides is a class of compounds known as organic phosphates. All of these have essentially the same mechanism of action and owe their effects to an ability to inhibit cholinesterase, the enzyme which normally detoxifies the excess of acetylcholine in the body. Acetylcholine is the chemical mediator formed at certain nerve-muscle junctions and the effects of the organic phosphates resemble it closely. They phosphorylate cholinesterase, causing irreversible inhibition. The first of these was synthesized by Schrader in Germany around 1940, and since that time these compounds have gained wide acceptance as pharmacological tools, chemical warfare agents, cholinergic agents in therapy, and as insecticides. The organic phosphates which are most widely used at present are the thiophosphates which are not active *in vitro* but are converted *in vivo* to the oxygen analogue, an active agent.

The organic phosphates are acutely toxic to mammals and even more toxic to fish. Table I (page 137) lists the LD_{50}'s for male rats of the organic phosphates used as pesticides. There are sex differences; usually females are more sensitive.[4] Based on calculations from animal studies and on data from accidental deaths, the lethal dose of most organic phosphates to man is less than one gram. A few like malathion (*O*,*O*-dimethyl dithiophosphate of diethyl mercaptosuccinate) are less toxic due to rapid enzymatic detoxification. The usual clinical symptoms of poisoning are: first, weakness, unsteadiness, blurred vision, headache, and nausea; secondly, these may be followed by vomiting, abdominal cramps, diarrhea, salivation, lacrimation, profuse sweating, tremors, pinpoint pupils, and difficulty in breathing; and, finally, severe muscular fibrilations, convulsions, coma and death. The actual cause of death is respiratory failure.

Subacute Effects of Organic Phosphates

Subacute effects of organic phosphates occur from repeated small dosages of the organic phosphates. The observed symptoms are similar to those occurring in acute doses, but varying in degree depending upon the exposure. Subacute data from animal experiments are used in the establishment of safe tolerance levels on raw agricultural products. Measurements are made on the inhibiting effects of small dosages which are too small to produce gross symptoms, but which do show inhibition of the cholinesterase activity of plasma, red cell, and brain. This inhibition of cholinesterase activity is a very sensitive index of toxicity and the establishment of a "no effect" level is one criterion used in establishing tolerances for residues of pesticides on food products.

Since organic phosphates usually are rapidly hydrolyzed by the nonspecific esterases of tissues to toxicologically inert compounds, they do not present as great a problem (in terms of chronic effects) as the chlorinated hydrocarbon pesticides. However, demyelination of nerves and neuromuscular damage have occurred from chronic exposure to some organic phosphates when administered in sufficient doses. The chronic toxicological effects of these compounds depend on the rate of enzymatic alteration, and this alteration can vary, even rendering a compound more toxic. Some of the recently synthesized

Fitzhugh: Table I

ACUTE ORAL TOXICITY OF CHOLINESTERASE INHIBITORS
(MALE RATS)

COMPOUND	LD_{50} mg/kg*
PHOSDRIN	6.1
SYSTOX	6.2
OMPA	9.1
PARATHION	13.0
GUTHION	13.0
METHYL PARATHION	14.0
TRITHION	30.0
EPN	36.0
DELNAV	43.0
DIAZINON	108.0
MALATHION	1375.0

*Gaines, T.B., TOX. and APP. PHARM. 2 88-99, 1960 compounds have been halogenated, and these show other types of chronic damage in addition to anticholinesterase effect.

Combined Effects of Pesticides

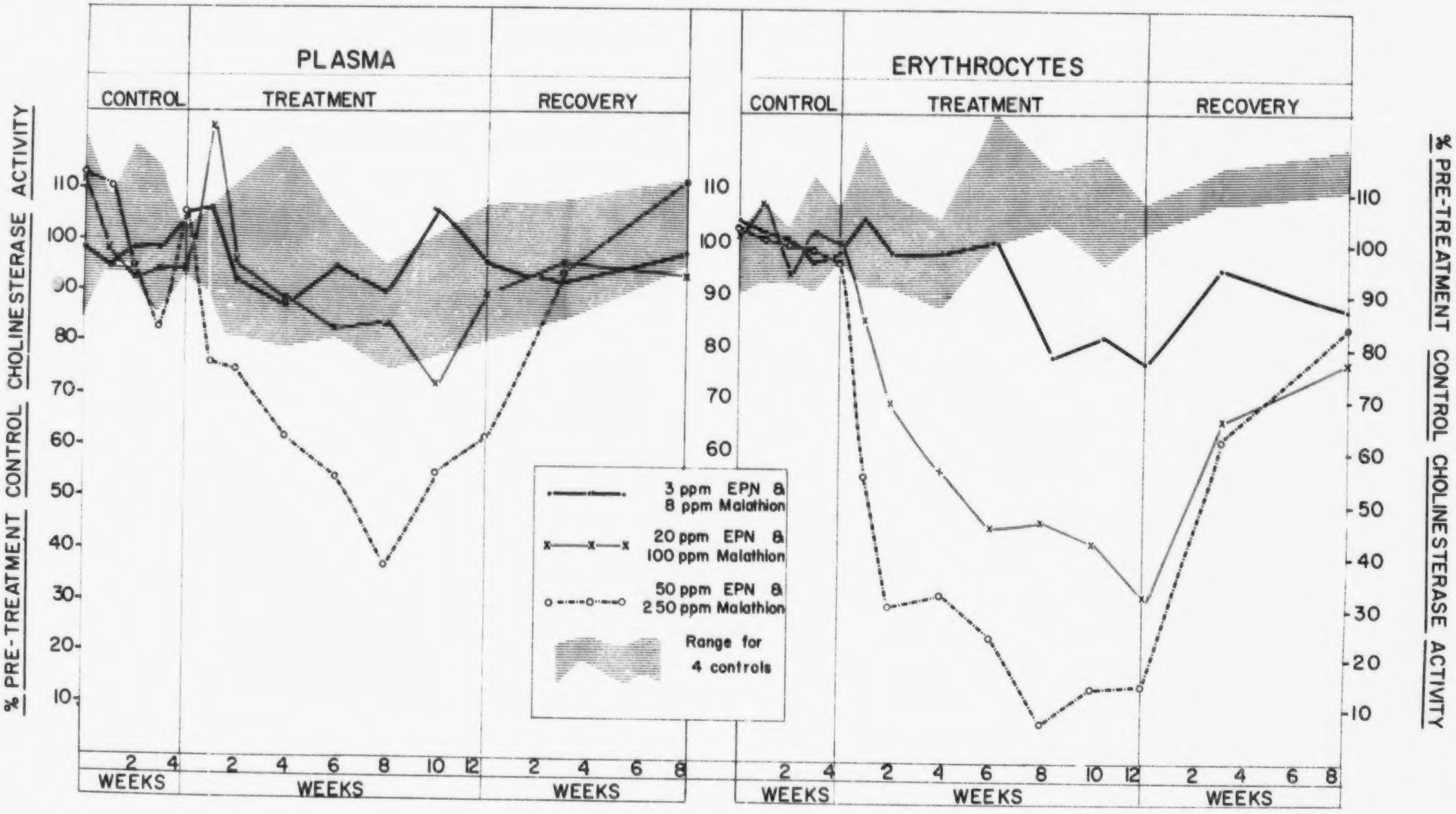
Another question which is often raised concerns the hazard of the presence of two or more pesticides on the same food product. We have made some studies in our laboratory on the combined effect of two organic phosphates on experimental animals. Frawley and associates[3] reported that when malathion and EPN were administered simultaneously to dogs there was a potentiation of toxicological effects (Fig. 1, page 139). More recently, our observations were confirmed by Murphy and associates[6] who reported potentiation between malathion and triorthotolyl phosphate. Cook et al.,[1,2] on the basis of *in vitro* work, showed that EPN inhibits the enzymatic hydrolysis of malathion, thus preventing its detoxification.

Until recently, only the potentiating effect on simultaneous administration was studied. Recent studies by Hagan[5] have shown that two organic phosphates, or a combination of an organic phosphate plus another cholinesterase inhibitor, such as Sevin (1-naphthyl-N-methylcarbamate), are more toxic if given consecutively than if given simultaneously. Table II (page 140) compares the lethal effect of the simultaneous administration of Delnav (70% cis and trans isomers of 2,3-dioxanedithiol-S,S bix (0,0-diethyl phosphorodithioate)) and malathion (*O,O*-dimethyl dithiophosphate of diethyl mercaptosuccinate) with that observed when Delnav was administered four hours prior to malathion.

The enhancement of the lethal effect by the prior treatment was approximately seven times. Table III (page 141) shows a similar enhancement of the lethal effect of two anticholinesterase compounds by the prior administration of an organic phosphate to a carbamate. The effect of the time of the prior administration on the onset of symptoms is shown by Table IV (page 142). The maximum potentiation was observed when Trithion (0,0-diethyl S-p-chlorophenylthiomethyl phosphrodithioate) was given four to five hours prior to malathion.

The above data illustrate that the combined action of more than one individual pesticide may increase the toxic effects, and complicates our evaluation of the safety of pesticides in food and water. As the number of contaminants increases, the problem of appraising accurately the toxicological hazards of pesticides in food and water becomes increasingly complex.

EFFECT OF DIETS CONTAINING MALATHION AND EPN ON BLOOD CHOLINESTERASE OF DOGS



Fitzhugh: Fig. 1

Fitzhugh: Table II

POTENTIATION DELNAV PLUS MALATHION

DELNAV mg/kg	MALATHION mg/kg	4 HOUR	SIMULTANEOUS ADMINISTRATION
50.0	1175	10/10	10/10
25.0	588	10/10	8/10
12.5	294	9/10	2/10
6.3	147	10/10	0/10
3.1	74	8/10	0/10
1.5	37	0/10	0/10
0.75	18.5	0/10	0/10

DELNAV	LD_{50}: 100 mg/kg	2.6	17.8
		+	+
MALATHION	LD_{50}: 2350 mg/kg	60.2	420.0

Fitzhugh: Table III

POTENTIATION DELNAV PLUS SEVIN

DELNAV mg/kg	SEVIN mg/kg	4 HOUR	SIMULTANEOUS ADMINISTRATION
118	470		10/10
59	235	10/10	6/10
29.4	118	9/10	2/10
14.7	59	1/10	0/10
7.4	29.5	0/10	
DELNAV	LD_{50}: 122 mg/kg	20.8	47.8
SEVIN	LD_{50}: 500 mg/kg	83.3	191.0

Fitzhugh: Table IV

POTENTATION OF SYMPTOMS

Trithion (5.2 mg/kg) given prior to Malathion (395 mg/kg)

Time Difference hours	Onset of Symptom minutes	Mortality
0	116	3/6
1	57	5/6
2	20	6/6
3	28	6/6
4	11	5/6
5	11	6/6

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PHYSIOLOGICAL EFFECTS OF INSECTICIDES ON AQUATIC LIFE

By

C. H. Hoffmann

From the viewpoint of public health and fish and wildlife resources, there is much concern about the water pollution that may result from land-management practices leading to siltation in streams, improper treatment of industrial chemicals before discharge, mine wastes, radioactive wastes, raw sewage, household detergents, and insecticides.

The widespread use of insecticides has received criticism as a water pollutant, and it is timely to review this matter since all of us have keen interest in clean waters and in the preservation and harvest of fish and wildlife. Entomologists, in response to grower and public demand, have the responsibility for developing and recommending ways to control destructive insects. As the most feasible approach for controlling most insects is the use of insecticides, the problem immediately resolves itself into the selection of those insecticides that are the most effective against destructive insects and at the same time the least hazardous to other organisms.

For several years I had an opportunity to study the effects on both terrestrial and aquatic insect populations of aerial applications of DDT over forests. It was soon determined that 1 pound of DDT per acre was a greater hazard to aquatic organisms, and accordingly studies were intensified on streams and lakes.

Early Experience Reviewed

In this paper I will review only the field studies made in this country and Canada since Rudd and Genelly (1956) published their bulletin on the effect of pesticides on wildlife. They concluded that pesticides were less harmful to fish and wildlife than were changes brought about by land and water development. Their bulletin revealed that few extensive experiments had been made along these lines. The few recorded losses to fish and aquatic birds were generally associated with the misuse of insecticides, such as cleaning of spray equipment in ponds or streams or their use on an emergency basis. Such was the record up to 1955 in relation to the

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treatment of millions of acres of crops, forests, and swamps that had been successfully sprayed during a 10-year period to control serious pests.

Need For Insect Control

Before considering the hazards of insecticides to aquatic life, it seems appropriate to evaluate the need for insect control. Let us not forget that insects are probably man's greatest challenge to supremacy on earth. In the United States about 10,000 species are injurious and, in spite of many effective control measures, they cause losses of approximately $4 billion annually. Without control, the losses would exceed this figure by several times. In fact, many crops could not be grown economically and our standards of health and economy would suffer serious setbacks. Insects are therefore of great importance and have a direct or indirect effect on each citizen.

Entomologists welcomed the availability in 1942 of DDT for the control of insect-borne diseases threatening military and civilian lives, which historically have been greater killers of man than the instruments of war. After the war DDT and other chlorinated hydrocarbon insecticides became available for civilian use. Within a few years outstanding control was obtained of insects that damage or destroy fruits, vegetables, tobacco, sugar beets, sugarcane, cereals, cotton, pastures, ornamentals, livestock, and forests. Soon the yields of many crops jumped 20 per cent or more, and they were of higher quality. Moreover, some of these new materials were effective against insects that carry destructive diseases of plants, livestock, and man. Other new types of insecticides were also developed, including organic-phosphorus compounds, carbamates, and synthetic pyrethrum-like materials. Crop losses caused by many pests were reduced drastically.

Recommended Applications of Insecticides

This [illegible] the background that led to our great dependence on insecticides. For several years now the Department of Agriculture has issued a handbook containing insecticide recommendations of the Entomology Research Division (1960) for the control of insects attacking crops and livestock, which also includes precautions, safeguards in relation to fish and wildlife, and restrictions to protect spray applicators and also to meet tolerances established by health officials.

The airplane is an accepted tool of farmers, foresters, and public health workers for the rapid dispersal of insecticides. The importance of custom applicators should be recognized, and every assistance given to them to achieve control with safety to all. In 1957 about 4,000 pilots sprayed approximately 46 million acres of land in the United States for control of insects, including such agricultural pests as the boll weevil, the imported fire ant, the Mediterranean fruit fly, the Japanese beetle, grasshoppers, and mosquitoes, and such forest insects as the spruce budworm and the gypsy moth.

The increased use of insecticides has led to greater chances of water contamination through drift and runoff from agricultural land. Let us now look at some of the results of research on damage to aquatic life resulting from insecticide applications since 1955.

Effects of Chlorinated Hydrocarbon Insecticides - DDT

Much publicity has been given to a substantial fish kill several months after the aerial spraying in 1955 of forest land near the Yellowstone River in Montana. Since there was no other evident cause, this fish mortality was attributed by some to DDT spraying at the rate of 1 pound per acre for control of the spruce budworm. Because there was no mortality at the time of spraying (Parker 1956), and of the distance of the forest from parts of the river, DDT could hardly have been the cause of such delayed mortality of large fish (Hoffmann 1958, Hoffmann and Drooz 1953, Cope and Springer 1958).

Cooperative studies on 13 smaller streams in this area in 1956 by the U. S. Forest Service, the U. S. Fish and Wildlife Service, and the Montana Fish and Game Department confirmed previous findings in the East (Hoffmann and Drooz 1953) that many aquatic insects were killed by DDT spray dispersed from airplanes at 1 pound per acre (Graham and Scott 1958). Most of the trout gorged on these insects but did not succumb, and there was a gradual repopulation during the 4 months after the spraying, and no indication of leaching of DDT into one stream after a 1.2-inch rainfall. Other findings of note were: (a) Numerous yearling brook trout collected in one stream a year after treatment showed survival of young-of-the-year fish; (b) live brook trout captured after 16 months contained DDT; and (c) trout in a sprayed stream where the bottom fauna was depleted, fed on terrestrial insects and midge fly larvae. In 11 of these small streams, in which maximum damage would be anticipated, there was no delayed fish mortality, but in two of them game-fish reductions of about 70 and 80 per cent were found in certain sections which may have been due to delayed effects of DDT.

In 1957 a more intensive study was made on the Ruby River in Montana following airplane spraying of adjacent forest at the rate of 1 pound of DDT per acre (Graham and Scott 1959). Samples of surface water taken 5 minutes after the spraying contained 1.35 ppm, whereas samples taken 32 hours later revealed only a trace. None was found in samples taken following two heavy rains after the spraying. Though no DDT was found in bottom sediments, it was found 2 months later in aquatic vegetation 10 miles below the sprayed area. Bottom insects showed slight recovery in 1957 and considerable recovery in 1958. Greatest mortality of suckers, the predominant fish, occurred in the first 2 weeks, and there was some kill throughout the summer and fall. Dead brown trout were found below the treated area after 5 months.

Observations on two other rivers indicated marked losses of aquatic insects but no fish mortalities. Additional studies are needed to explain the wide discrepancies on physiological effects observed on animals in streams that have been treated with DDT at the same dosage.

In 1958 over 300,000 acres of spruce-fir forest in Maine were aerially sprayed with 1 pound of DDT per acre to control the spruce budworm. Blocking nets were operated in 13 brooks for 170 net-days after the spraying. Of 8,844 dead fish, 216 were brook trout, 65 being young-of-the-year (Warner and Fenderson 1960). Suckers, minnows, sculpins, and sticklebacks appeared to be most affected. Dead trout collected immediately after the spraying contained 4 to 26 ppm of DDT in their body tissues; live trout collected 3 months later contained 3 to 198 ppm. In some of the brooks there was a significant growth increase in small trout, possibly because of the reduced population. The number of bottom insects that serve as trout food varied in the different brooks, and in some the trout utilized snails and terrestrial insects. There was no evidence of trout starvation as a result of an inadequate food supply. A strong 1959-year class of trout was responsible for rapid population recovery by the fall of 1959, and it was believed that an equally strong 1960-year class would assure recovery to prespray levels.

Of considerable concern were the results of studies made by Canadian workers (Canada Departments of Agriculture and Fisheries, etc., 1958) on salmon showing drastic reductions in the smallest age classes in some DDT-sprayed streams in both New Brunswick and British Columbia, since it was feared that there would be a serious reduction in the adults returning from the sea.

In 1954 about one-third of the Miramichi River drainage in New Brunswick was aerially sprayed with DDT at ½ pound per acre to control the spruce budworm. Large numbers of fish up to 2½ inches in length were killed directly by the spray, and lack of aquatic-insect food after the spraying may have contributed to the mortality of small salmon later in the season. Ide found that the numbers of insects increased within 3 months but the quantity was one-half to two-thirds of that before the spraying. Caddis flies and other insects important in the diet of small salmon were eliminated in some sections of the streams. According to Keenleyside, when spraying is not repeated over the same area for several years, the salmon populations appear to recover. However, most of the Miramichi watershed has been sprayed two or three times in the last four years, and since most of these salmon spend 3 years in fresh water before going to sea, some age classes have been subjected to more than one spraying. Kerswill and Elson expected that by 1961 the adult trout stock would be only 25 per cent of the normal level prior to 1955, and that, because of the many factors affecting fishing success, decreases down to 50 per cent of normal might not be apparent in angling and other catches. It is gratifying to note that in 1958 and 1959 good runs were reported in some of the streams first affected by the spray, coincident with improved catches that were general for the species throughout its Atlantic range (Webb 1959). Thus, the affected streams seem assured of prompt and adequate restocking. Otherwise, as suggested by Kerswill and Elson, the harmful effects of the spraying presumably can be overcome by planting hatchery stock and transplanting stream insects.

In 1957, 155,000 acres of timber on Vancouver Island were sprayed with DDT at 1 pound per acre to control an outbreak of the black-headed budworm. Bottom organisms in streams were sampled, and fish were exposed in live pens in streams that purposefully were not directly sprayed. According to Hourston, mortality of Coho fry ranged from 0 to 91 per cent; fingerling mortality was less. Loss of bottom organisms paralleled closely the loss of fish. Though loss of trout fry was sometimes great, angling pressure is relatively light and the stocks should repropagate quickly. However, 2-year classes of the anadromous steelhead trout were affected, and it will take a number of years for them to overcome the detremental effects of the spray.

Effects of Chlorinated Hydrocarbon Insecticides - BHC and Strobane

In 1955, BHC was applied in a wettable-powder suspension at ½ pound of the gamma isomer per acre on a fresh-water lake in Florida to control immature stages of May flies. Swarms of adults were so obnoxious that residents threatened to sell their homes and move away unless relief could be obtained. The treatment was effective in killing the May fly nymphs and apparently had no effect on fish or other aquatic life (Lieux and Mulrennan 1955).

In 1955 a study was made in Delaware to determine the effect on wildlife of various insecticides used for mosquito control on tideland areas (George, Darsie, and Springer 1957). Gamma BHC, DDT, and Strobane in oil solution were applied simultaneously by airplanes to similar habitats at rates of 0.1, 0.2, and 0.3 pound per acre. There was little difference in the deaths of estuarine fish and blue crabs between treated and control plots, or among the three treatments. Both field observations and crab-pot counts showed that the fish and blue crabs avoided areas of high insecticide concentration. However, the marsh fiddler crab was greatly reduced in numbers by all the treatments.

Chlorinated Hydrocarbon Insecticides - TDE

A hazardous food-chain relationship has shown up in connection with the treatment of Clear Lake (about 42,000 acres) in California with TDE to control the Clear Lake gnat, a serious nuisance to residents and to tourists who enjoy vacationing and the excellent fishing on that lake. The Entomology Research Division had an important part in developing the use of TDE for almost complete (99 per cent) control of this pest with no apparent damage to fish, which was considered an outstanding accomplishment. Now, according to Hunt and Bischoff (1960), repeated treatments of the lake may be the indirect cause of the death of hundreds of western grebes. This is amazing since the initial treatment was made at 1 part of TDE to 70 million parts of water. Dead grebes were found after each treatment, and chemical analyses of fatty tissue indicated about 1,600 ppm of TDE. Food-chain organisms were suspected. However, sufficient plankton samples could not be obtained for chemical analyses, and the presence of TDE was not established. Visceral fat from bullfrogs contained 5 ppm of TDE.

Since the grebes began to die 1 or 2 months after each treatment, it seems likely that they died of chronic poisoning. Most of the insecticide was believed to have been assimilated from contaminated fish and insects. In general, small fish accumulated less TDE than larger fish, and plankton eaters less than carnivorous fish of the same size. Analyses of visceral fat from large, apparently healthy large-mouth bass and white catfish, indicated accumulations of TDE as high as 2,275 and 1,700 ppm, respectively. Hunt and Bischoff suggested two possibilities in the event further TDE treatments are made: (a) Some species of fish might become resistant to TDE and not be affected by small dosages beyond the normal toxic threshold; and (b) conversely, they might add enough TDE to that already accumulated to prove lethal.

Effects of Chlorinated Hydrocarbon Insecticides - Toxaphene

In 1959 the U. S. Bureau of Sport Fisheries and Wildlife studied the effects on wildlife of 1½ pounds of toxaphene per acre used to control grasshoppers in Montana. No fish were present in a stock pond, but some salamanders, frogs, turtles, and snakes were killed. Surviving frogs and turtles showed a loss of coordination; however, the turtles were wary 12 days following treatment (DeWitt and George 1960).

Effect of Chlorinated Hydrocarbon Insecticides - Heptachlor

In the Federal-State program that has been carried out in the Southern States for the eradication of the imported fire ant, heptachlor granules have been used at the rate of 2 pounds per acre. In Hardin County, Texas, 1,400 acres were treated. Canal banks were hand-treated to protect fish, but some of the insecticide blew into the canals. As recorded by Lay (1958), fish and other aquatic forms were dying after 14 days. Though some live fish and crayfish were present in cages, seining indicated smaller populations than in pretreatment samples.

In 1958 R. A. Hoffman, of the Entomology Research Division, made some incidental observations (unpublished) on fish in a 900-acre area in Montgomery, Alabama, in which the pasture portion contained a shallow 1/6-acre pond and a deep (maximum 6 feet) 2½-acre farm pond, both with warm-water game fish. The airplane pilot endeavored to avoid direct treatment of the large pond but not the smaller one. Much of the land drained into the large pond. Dead fish, mostly stunted bluegill sunfish, were found on the surface of the shallow pond, but none in the deep one.

DeWitt and George (1960) have reported on the effects of these treatments in several States. In Wilcox County, Alabama, some adult fish were killed soon after treatment (presumably in 1957) of only a portion of a 2-acre pond; however, small fish were observed through 1958, and 68 pounds of fish, representing 10 species, were found alive in the fall of 1959 when the pond was drained. Observations in Decatur County revealed no damage to fish. In Louisiana there was some damage to fish in shallow water in some localities and minor losses in others.

The treatment had a variable effect on frog populations, depending upon species and habitat. Mortality occurred in some areas and hardly any damage in others. Ground dwellers were more severely affected than tree frogs.

In Wilcox County, Alabama, 137 cottonmouth snakes were captured, marked, and released during the first year after treatment of the area with heptachlor. Recaptures of these snakes steadily declined until 15 months after treatment, after which no moccasins were found in the study area. Snakes of the genera Storeria and Natrix suffered the same fate (DeWitt and George 1960). Though quantitative data on population densities of aquatic turtles in the same area are lacking, it is believed that they were virtually eliminated.

Inasmuch as the dosage of heptachlor granules is now being reduced to ½ pound per acre, with two applications 6 months apart, it is believed that future fish losses will be avoided.

Effect of Chlorinated Hydrocarbon Insecticides - Dieldrin

Harrington and Bidlingmayer (1958) studied the effects of dieldrin on fish and invertebrates in shallow ditches within a 2,000-acre salt marsh in St. Lucie County, Florida. The county government had made airplane applications of dieldrin granules at the rate of 1 pound of toxicant per acre to eliminate sand flies, which cause severe annoyance to people living near marshes. The fish kill was very heavy, and by extrapolating data from the two ditches the authors estimated that 20-30 tons of about 30 species were killed. However, by the fourth week after spraying there was an increase in killifishes, and by the eighth week some species were abundant. Mollusks appeared unharmed by the spray, but most of crustaceans and crabs were eliminated.

In 1954 a cooperative study was undertaken by the U. S. Department of Agriculture and the Public Health Service to determine the toxicity of runoff waters from hospital grounds treated with dieldrin at the rate of 4-2/3 pounds per acre to control whitefringed beetles in Alabama. Bioassays of runoff water following the first rain after the treatment, which drained from the grassy slope into a storm sewer, showed it to be toxic to fathead minnows at a dilution of 1 to 3 (Tarzwell and Henderson 1957). The runoff was estimated to contain 0.128 ppm of dieldrin. Apparently there was no opportunity for the insecticide diluted by rain to pass over untreated soil before reaching the storm sewer.

Effects of Organic-Phosphorus Insecticides

In 1956 malathion sprays--2 pounds of a 25- per cent wettable powder plus either 1 pound of yeast hydrolysate or 1 quart of sauce base No. 2 in 1 gallon of water per acre--were applied repeatedly to eradicate the Mediterranean fruit fly from 800,000 acres in Florida. In certain areas, especially in shallow water, large numbers of killifishes and certain other species were killed by these sprays (Cope and Springer 1958). There was also some loss of tropical fish, but the fish losses were considered minor in relation to the large spray area and the need for repeated spraying until this fruit fly was eradicated.

There has been a surge in the development and use of organic-phosphorus insecticides, as indicated by the materials now in use (Metcalf 1959). Though they are highly toxic and great concern has been expressed regarding their use, experiments have shown that certain formulations can be used safely in the small amounts required for control of many insects. In aquarium and trough tests some have proved far less toxic to fish than several of the chlorinated hydrocarbon insecticides.

Little is known regarding the effects of many of these materials on fish and bottom organisms following field usage. The general-purpose residual insecticides in this group include Chlorthion, Delnav, Diazinon, dicapthon, Dipterex, EPN, ethion, Guthion, malathion, methyl parathion, parathion, Phenkaptone, Phostex, Potasan, sulfotepp, and Trithion; the contact insecticides include DDVP, Dibrom, and TEPP; the plant systemic insecticides embrace demeton, dimefox, dimethoate, Di-Syston, methyl demeton, phorate, Phosdrin, phosphamidon, and scharadan; and the animal systemics include Co-Ral and ronnel (Trolene).

Effects of Carbamate and Other Insecticides

Sevin (1-naphthyl methylcarbamate) has been found effective against grasshoppers and certain insects affecting cotton, fruit, vegetables, and forests. High levels of Sevin fed to cattle for sustained periods did not result in secretion in the milk. This insecticide is effective against the gypsy moth and may fill an important place in control programs where DDT cannot be applied without the hazard of contaminating adjacent pastures because of drift. The apparent low toxicity of Sevin to fish suggests its possible use in areas that include important fishing streams. However, research should be undertaken in the field to evaluate its effects on fishfood organisms and fish.

Other carbamate insecticides--Isolan (5-(1-isopropyl-3-methylpyrazolyl) dimethylcarbamate), dimetan, and Pyrolan (5-(3-methyl-1-phenylpyrazolyl) dimethylcarbamate)--and also barthrin, Tedion (2,4,4',5-tetrachlorodiphenyl sulfone), and Thiodan (6,7,8,9,10,10-hexachloro-1,5,5a,6,9,9a-hexahydro-6,9-methano-2,4,3-benzodioxathiepin-3-oxide) have come into use but have not yet been evaluated as to their effects on fish and wildlife.

Overall Effects of Insecticides on Aquatic Life

Since insecticides are hazardous to aquatic life, every effort should be made to avoid the contamination of ponds, lakes, and streams. However, these harmful effects are minor in view of the vast quantities used. One cannot help being impressed by the tremendous biotic potential of wildlife and the rapid recovery of species following temporary losses. Most of the large-scale insect-control operations are under the supervision of competent Federal and State authorities who have carefully weighed their benefits against other values.

Some of the fish kills reported have resulted from carelessness in application. Steps should be taken to prevent such situations, but occasional minor or temporary losses are to be expected in large-scale operations. Except for DDT, little is known about the effects on fish and fish-food organisms of insecticides that have been used for a number of years. However, millions of acres in this country have been successfully treated to control or annihilate insect pests without permanent damage to other interests.

Insecticides in Water Supplies

DDT has been recovered in amounts ranging from 0.001 to 0.02 ppm from rivers, including the Detroit, Mississippi, Missouri, and Columbia. According to Nicholson (1959), the amount recovered from treated municipal water is too small to affect humans, and actually constitutes a minor proportion of their exposure to the insecticide. Workers in water pollution believe that when insecticides are not present in sufficient quantities to kill fish, they will not constitute a public health hazard in drinking water. Rotenone and toxaphene can be removed from water during the purification process by use of activated carbon.

Present and Future Research

The Entomology Research Division recognizes the benefits and limitations of many control methods and is striving to solve the most urgent insect problems. Emphasis is being placed on safer chemicals and on methods of using them with maximum safety. Research on biological-control organisms is being stressed where this type of control appears useful, either alone or in combination with insecticides. Additional crop varieties resistant to insect attack need to be developed. Basic studies are being undertaken on the possible use of selective chemicals, such as hormones and growth-inhibiting substances.

New ways will be sought to use insecticides, as in baits, in order to lessen the amount of toxicant required. Advancements are being made in the development of systemic materials that are specific against certain pests, and of new formulations and materials that are effective against pests but less toxic to other animals. A unique new approach which leaves no residues or other undesirable effects, involves the continued release of males sterilized by gamma rays from a cobalt-60 source to destroy the reproductive potential of natural populations. This technique has eradicated the screw-worm from the Southeast and may be useful for the control of other insects, but not until after considerable further research.

There is a marked need for broad cooperative studies between entomologists, fishery biologists, and chemists to determine the immediate and long-term effects on aquatic life of insecticides in current use and those expected to be used in future control programs. Apparently there have been no detailed studies on effects of insecticides on fish-food organisms except for DDT.

Some laboratory experiments have been made on the comparative toxicity of new materials to fish, but the real test comes in evaluating them under field conditions where many variables are operative. Among these variables are (1) ecological differences in ponds, lakes, and streams in different parts of the country; (2) many aquatic insects with different habits; (3) many species and age classes of fish and their susceptibility to poisoning; (4) shore line or tree canopy; (5) proximity to untreated water to furnish immigrants for repopulation; and (6) influence of weather on the application and distribution of the insecticide.

Field Experiment Data

In field experiments there must be suitable controls and pretreatment samples. The greatest lack thus far is of quantitative data to relate observed or measured mortality to the total population for each animal studied. Care should be taken in extrapolating results obtained on small study areas to larger acreages. The relationship between the abundance of bottom organisms and fish populations should be determined.

We know that fish have food preferences and also that they are opportunists. Thus, a considerable but temporary loss of bottom organisms may be of little importance. After a spraying fish will feed on terrestrial insects falling on the water and on those bottom organisms that survived the spray even through they might not be normally utilized. On the other hand, certain treatments might cause heavy losses of aquatic-fish foods that would be replaced very slowly and thus affect the condition of the fish.

We need to know more exactly what happens when a lake or stream receives an insecticide spray. How much insecticide reaches the water, and how long can it be detected in water samples? Does it settle out in the mud or on bottom vegetation, or is it blown to the shore in an oil film? If absorbed on mud or vegetation, is it likely to poison small animals or fishes? Are fishes killed from direct contact with the spray or from feeding on spray-killed insects, or both? Why do some fishes that gorge on treated insects survive whereas others die?

Transient Vs. Continuing Effects

Are these effects merely transient, or can important fish losses occur long after a spraying because of insecticide accumulation on vegetation many miles downstream from the area treated? I am convinced that the turbidity and high organic content of water can nullify the effect of DDT on fish. A study of this matter might lead to ways of introducing a material into a stream or artifically muddying the water for a brief time during the spray operation to curtail the toxic effects of the insecticide on fish.

An evaluation should be made of insecticide runoff from cultivated fields and orchards, with consideration of different degrees of breakdown in different soil types, rainfall intensity and land cover, and absorption by the soil. As yet no one has demonstrated sufficient runoff from forests into streams to show a measurable increase in the mortality of bottom organisms above that caused directly by the spraying.

Research to Minimize Hazards

Research is needed to develop better formulations and equipment for dispersing sprays of suitable particle size to minimize losses of toxicant, to increase insecticidal efficiency, minimize hazards to wildlife, and avoid removal by rain from the target plant. Improvement of operational procedures, especially for aerial spraying, might well reduce the hazard of insecticides to aquatic life. There is still a need for a manual to cover the many problems that arise in connection with any large-scale aerial spray program. A manual based on the best procedures developed through the years of experience by successful pest-control operators would serve as a helpful guide for the conduct of future effective and safe programs.

Summary

Insects are probably man's greatest challenge to supremacy on earth. DDT and other synthetic organic insecticides have saved millions of lives by controlling insects that carry diseases; have greatly increased the yields and quality of food, feed, and fiber crops; and have saved forests from destructive insects. Larger quantities are required each year, and the use of airplanes to disperse them is increasing. The widespread use of insecticides concerns the public because of their hazard to health and of damage to fish and wildlife.

Insecticides cause only minor losses of aquatic life in comparison with the vast quantities used. Other factors in land use and development appear to be more damaging. Some losses attributed to insecticides are due to misuse and cleaning of spray equipment in ponds and streams. Most large-scale spray operations are under the supervision of competent Federal and State authorities, who have carefully weighed the economic benefits of controlling or eradicating serious pests against other values, including those associated with water resources.

The Entomology Research Division recognizes the benefits and limitations of different control methods and is striving to meet the most urgent problems. Research is being undertaken on biological control, the development of additional crop varieties resistant to insect attack, use of hormones and growth-inhibiting substances as control agents, new ways to use insecticides such as in baits, systemics that are specific against certain insects, and the release of males sterilized by irradiation to destroy the reproductive potential of natural populations.

There is a marked need for cooperative studies between entomologists, fishery biologists, and chemists to determine the immediate and long-term effects of insecticides on aquatic life. Most of the studies thus far have related to DDT, and many of these have not been quantitative. Field studies are essential for a practical evaluation of each insecticide on aquatic life and, more important, for the development of sound control recommendations with minimum damage to aquatic life.

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OPEN DISCUSSION

DR. GAUFIN: Since Dr. Hoffmann did not elaborate on one item noted in his paper, I wish to present additional information relating to it before we take up questions.

In the Yellowstone River Study which Dr. Hoffmann mentioned, it was estimated that 95 per cent of the aquatic insects along a 100-mile section of the stream were killed, and that losses of trout, whitefish and suckers occurred in early October. Certainly, with that loss of aquatic insects, this loss of fish after three months, might be attributed to starvation.

I would like Dr. Hoffmann to comment later on the condition cited. I am sure all of you are acquainted with the fact that there is a considerable amount of literature as to the toxicity of various insecticides to fish under experimental conditions. In fact, at the Robert A. Taft Sanitary Engineering Center, a considerable amount of work has been done indicating that the toxicity of insecticides to bluefills, guppies, and several other species of fish is rather severe. I think they have found that concentrations of considerably less than 1 ppm are toxic to experimental fish in the laboratory.

In some work I have done in studies of aquatic insects at the University of Utah, for example, I have found concentrations as low as one part per billion are toxic to certain aquatic insects. It should be recognized, of course, that conditions in the laboratory are considerably different than those in the field. Nevertheless, low concentrations of insecticides may cause damage.

In fact, if we had fisheries and wild life people here to present papers on this subject I think we might have a little different side of the picture presented. I think the picture isn't bright and I am sure some of you agree with me. Dr. Weiss has been doing work to determine the effects of insecticides on fish, and might have a few pertinent comments to offer.

DR. WEISS: Some of you are aware of the type of studies we have been making. We have been investigating not the direct toxic effect of insecticides on fish, but the effect on specific enzyme systems - in this case the brain cholinesterase of fish. We are not interested in killing the fish, per se. Of course, we have to remove the brain to assay it, but by using the brain tissue as a rich concentrated source of nerve tissue, we can determine the degree of inactivation of cholinesterase after exposure to insecticides.

The studies we have done confirm the results obtained by direct toxic exposures in studies at the Sanitary Engineering Center; particularly in that the species differences are rather exaggerated. The species differences can be followed both in the patterns of inactivation of the brain cholinesterase as well as in the pattern of recovery.

We can follow the extent and rate of recovery of the brain enzyme after exposure to enzyme inactivators by removal to non-contaminated water. The time of recovery curve appears to be expressive of both chemical and biological species. The chemistry of the particular compound, the physiology of the fish species, as well as the mechanisms of molecular transfer through membranes of the fish, all become part of the total expression of toxic response.

In recent studies, we have extended our exposure conditions to utilize the raw water supply of Chapel Hill, North Carolina. We tap off from the water supply before it enters the treatment plant, and discharge the raw water into our exposure vessels. At this point we introduce organic phosphororous insecticides in extremely low concentrations. Currently, we are working in the range of .01 mg/l. The compounds tested to date have been parathion, EPN, and gluthion. In about two days the brain cholinesterase activity drops below the normal range, and in approximately ten days reaches a point of 40 to 50 per cent of normal activity. At this point it appears to come into an equilibrium and not drop further. However, we have not carried out experiments of sufficient duration to confirm this. The important aspect of these experiments is that a direct physiological effect is evident even at these very low concentrations.

Although the dose levels employed are not hazardous to man, the technique is capable of detecting the presence of these compounds in such low concentrations in water. Perhaps of somewhat more significance is the fact that fish exposed to these compounds and then removed to non-contaminated water require a minimum of ten days to two weeks before the brain enzyme activity returns to normal. Thus, fish exposed to contamination are distinctly marked for this period. When enough information on biological and chemical species relationships has been developed, it may be possible to extrapolate back in time and estimate the day on which exposure occurred.

DR. GAUFIN: I believe Dr. Hueper has questions to ask.

DR. HUEPER: I would like to ask Dr. Hayes whether it is proper to compare the death rates from leukemia with the death rates from agranulocytosis. Leukemia has a 100 per cent death rate. That is, morbidity rates and mortality rates are identical. But in agranulocytosis, during the last fifteen years, we have experienced a very high recovery rate. I know agranulocytosis very well and am one of the pioneers of that field of study in the United States.

There can be no doubt that a large number of drugs which produced agranulocytosis have been introduced into our medical field during the last twenty years. This is evident to anyone who reads the Journal of the AMA. There should be a large number of agranulocytosis cases. Naturally, most cases are not recorded and most of them will recover. So there can be no doubt that to compare the agranulocytosis mortality rate with the leukemia rate is misleading.

The second point is: Do agents with leukemogenic action, such as ionizing radiation, usually have a primary agranulocytotic phase? Recent reports in Germany indicate suspicion that quite a number of the agranulocytotic drug cases will later develop leukemia. Insecticides -- specifically, the chlorinated hydrocarbons -- have been considered a cause of agranulocytosis (and they have also been accused of causing leukemia in some cases). The existence of such relations is still an open question, and one cannot simply say these chemicals are not leukemogenic.

DR. HAYES: I think consideration of the best available statistics may mislead some people, but not very many. There is some advantage in using a definite end point -- in this case death rates -- on which we do have the figures.

Now, on the matter of the relationship between these two diseases, it is generally agreed that there is some difficulty in making a decision in early cases. But the question of whether or not they are truly related is a matter of theory in which there are certainly two different schools of thought. One holds that they are, and one that they are not. The fact remains that, of cases receiving these several diagnoses and going on to death, the official figures for this country are those illustrated in the chart (see page 165). There is no change in trend in the one which has 100 per cent mortality coincident with the introduction of the pesticides. Therefore, there is no objective evidence whatever that the pesticides have any influence on the trend.

DR. GAUFIN: I would like Dr. Hoffmann to answer the question of whether or not 95 per cent loss of aquatic insects in a stream would be considered important.

DR. HOFFMANN: I was not present at the kill referred to, so these comments will of course, be speculation. I think about all we can do in some of these situations, if we do not have data right on the spot, is to endeavor to arrive at our answer on the basis of past experience. In the Yellowstone River Study, so far as I know, no pretreatment samples were taken of bottom organisms, and that is a weakness. Fish kept in live pens at the time of treatment were not killed. Any evaluation certainly should have quantitative data, and we should have pretreatment samples to compare. Actually, no one has proven that DDT was the cause of losses in numbers of bottom organisms or fish in the Yellowstone River Study.

On the basis of several years' study in the East and some research in the West, the reason it seems to out of the ordinary to me, that DDT should be responsible was the fact that the forest was some distance from the stream. Theoretically any DDT that got in the stream, except for a little drift, would supposedly be contributed by runoff. I do not consider this source would be responsible, because it has never been demonstrated that spray runoff or leaching into streams occurs following the spraying of forests.

Second, the Yellowstone River is deep and swift. Subsidiary studies made two years later by the Forest Service and the Montana Fish and Game Department on small, shallow streams in which a maximum effect of DDT would be expected, did not result in extraordinary losses of bottom organisms and fish in most streams.

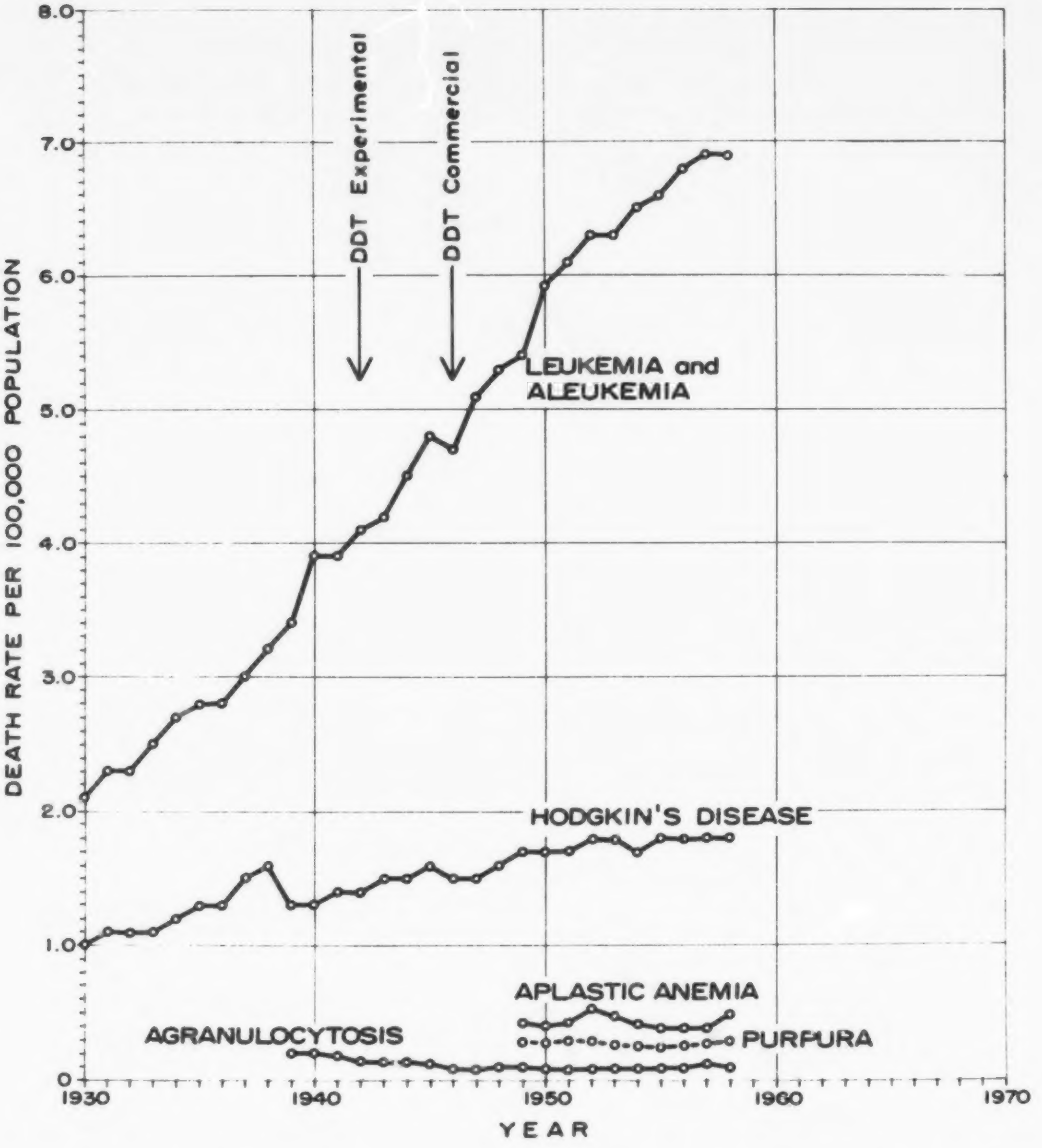

Figure 1. Death rates for selected causes, United States, 1930-1958
Source: "Vital Statistics of the United States."
DDT was introduced experimentally and commercially as indicated.
Other new pesticides were introduced at same time as DDT or later.

I would like to stress ecology of the streams because it is so important. We witnessed the treatment of a Wyoming stream, with DDT at twice this dosage rate, in connection with bark beetle control work. This particular stream was small and shallow; it had a series of beaver dams; and debris and a great deal of organic matter was present. On the basis of bottom samples there was only 50 per cent mortality of aquatic insects. Bottom samples are very indicative because insects are so sensitive. Thus, depending on the ecology of streams, very different effects may result.

Lately, I have been concerned about a difficulty some research workers experience in making stream studies - they are reporting DDT present in streams in areas not treated with DDT. This is disturbing; I do not know why these results are being obtained.

There is a real need, I think, to have presamples and to be able to follow experiments closely on a long-term basis. The many organisms involved make studies especially difficult. Considerable systematic entomology is required in making such studies, because the species vary in different streams and regions. It may be that some delayed fish kills will result from the use of certain pesticides, but I think no one has yet satisfactorily explained the actual factors that may be involved.

It would be quite interesting to know whether or not selenium might be involved in some of these fish kills that have been attributed to DDT. We know that many of the irrigation waters have a considerable content of selenium. We know it is toxic to animals. I am not aware of any specific information on the effect of selenium to fish.

DR. GAUFIN: May I express my appreciation to the speakers and participants in this discussion period.

MINIMAL EFFECTS OF ORGANIC WATER CONTAMINANTS

By

John A. Zapp, Jr.

While water can be defined in chemical terms as H_2O, we never find it in nature in its chemically pure form. Even rain water picks up contaminants as it falls through the atmosphere. Whether it comes from springs, wells, lakes or streams, it always contains both organic and inorganic contaminants placed there by its unique solvent action as it comes in contact with rock and soil and with living and dead organic material of animal and vegetable origin. If we want pure H_2O we must obtain it by distillation, and those who have drunk distilled water find that it has a rather flat and uninteresting taste. Distilled water also has a detrimental action on many aquatic organisms.

Water [illegible] Physiological Processes

Professor Lawrence J. Henderson some 47 years ago devoted considerable space in his book "The Fitness of the Environment" (1) to a discussion of the unique properties of water which are a necessary condition to life as we know it. He noted that: "Water is ingested in greater amounts than all other substances combined, and it is no less the chief excretion. It is the vehicle of the principal foods and excretory products for most of these are dissolved as they enter or leave the body. Indeed....it has become evident that the organism itself is essentially an aqueous solution in which are spread out colloidal substances of vast complexity. As a result of these conditions there is hardly a physiological process in which water is not of fundamental importance."

Professor Henderson concluded as follows: "Water, of its very nature, as it occurs automatically in the process of cosmic evolution, is fit, with a fitness no less marvelous and varied than that fitness of the organism which has been won by the process of adaptation in the course of organic evolution.

"If doubt remain, let a search be made for any other substance which, however slightly, can claim to rival water as the milieu of simple organisms, as the milieu interieur of all living things, or in any other of the countless physiological functions which it performs either automatically or as a result of adaptation."

The Potability of Water

The importance of water to us is beyond question. Yet so far as our use of it for drinking or cooking purposes is concerned, it is not a pure chemical substance. As defined by the U. S. Public Health Service Standards of 1946, (2) potable water has the following physical properties: "The turbidity shall not exceed 10 ppm, color 10 ppm, total solids 1000 ppm and odor and taste shall be absent."

A British report (3) defines potable water in somewhat similar terms: "As a chemically or physically pure water cannot occur in nature, purity implies pleasing to the senses, that is, absence of visible particles, turbidity, colour, taste and odour, and freedom from excessive amounts of substances in solution not normally detectable by the unaided senses. Potability, therefore, implies physical attractiveness as well as safety."

Contaminants in Water

We are here concerned with the physiological effects of organic contaminants of water, and what do we mean by this? I think that we are asking whether the dissolved organic materials which we find in treated and presumably potable water today are capable of exerting undesirable physiological effects. I do not believe that we are talking about gross or immediate effects but rather about minimal effects, difficult to detect except, perhaps after a long period of ingestion. At any rate, it is the minimal effects which I propose to consider.

Water, of course, has always contained organic contaminants. The organic burden of natural waters varies tremendously being perhaps minimum in clear cold spring water and maximum in relatively stagnant water of rivers, ponds, swamps and marshes. Even if the latter types of water are allowed to settle, the supernatant usually contains enough colloidal organic matter to impart color, odor and taste which civilized man finds objectionable.

Along with dissolved or suspended organic matter, natural waters may contain living forms such as bacteria and parasites which are definitely pathogenic to man - and which derive their nourishment at least temporarily from the aqueous environment in which they find themselves. One need mention only typhoid fever, cholera, dysentery and hookworm disease as examples of water-borne diseases. We have recognized these hazards and have learned to deal effectively with them, if only within the last century.

New Hazards From Organic Contaminants

Now we are concerned with possible new hazards from organic contaminants of water. These originate from the fact that man has created a great many organic compounds which are not found in nature and which are technologically useful.

Some of these new organic contaminants may appear in the waste waters of industrial plants. Some, like detergents, appear in household waste waters. Some, like agricultural chemicals and pesticides, may be washed from crops or leached from the soil by rainfall or irrigation. Some of these contaminants which appear in waste or run-off waters may find their way into sources of public water supplies and may appear in part in treated water derived from such sources.

There are a number of factors which tend to minimize the amount of organic contaminants in treated water. These are: (a) removal or destruction in waste or sewage treatment plants; (b) removal by oxidation or filtration as waste waters pass through rock, soil and sand; (c) removal or destruction as waters are held in impounding reservoirs; and (d) removal or destruction in water treatment plants. Not much in the way of organic contaminants is likely to pass this series of obstacles and turn up in treated water which meets the stated U. S. Public Health Service specifications for potable water.

Public Health Problem of Organic Contaminants

Nevertheless, I presume that some of these newer organic contaminants do turn up in small amounts in some treated waters and that this is the reason for their consideration in this Conference. It follows from their detection, identification and measurement that we must ask ourselves whether these new organic contaminants in the amounts present pose a problem with respect to the public health.

This problem does not differ from the related problem of small residues of organic contaminants in our food supply which has been the subject of much recent discussion and some new legislation, of which I am sure you are all aware.

Man lives in two kinds of environment. His external environment is variable - hot or cold, wet or dry. His internal environment is remarkably constant with respect to both composition and temperature.

And this constancy must be maintained while man exchanges materials and energy between his external and internal environment. When foreign substances are taken into the body, the internal environment must cope with them promptly by excreting them through the several channels available; or by converting them to harmless or even useful materials; or by storing them at least temporarily in a place where they will do no harm. If the body fails to do these things, bodily injury or death results and we call the capacity of a material to produce such bodily injury or death toxicity.

Significance of Toxic Effect

It is unfortunate but true that many of the technologically useful organic compounds which man has created through new combinations of elements possess toxicity as an unwanted and sometimes unsuspected attribute. It is important, therefore, that we understand the rules which govern the manifestation of toxic effects.

First, to exert a toxic effect, a poison must enter the body's internal environment. It is harmless so long as we can prevent it from getting on or into our bodies.

Second, the science of experimental toxicology has demonstrated that for each toxic material there is a necessary and sufficient amount which must enter the body before the toxic effect appears. If this amount is very small we call the chemical "highly toxic;" if large, we call it "slightly toxic" or "practically nontoxic."

Third, the toxic effect of a given material diminishes with decreasing dose and eventually disappears. In every experimental study of toxicity it has been possible to select doses so small that the characteristic toxic effect of the material does not appear within the normal life span even when the material is administered over that entire life span. This includes even the chemical carcinogens.

Physiological Adaptability

Perhaps these concepts can be illustrated by the analogy of drug action. If we have a simple headache when we know from past experience is amenable to aspirin, it will do no good to take a bottle of aspirin tablets from the medicine cabinet and just look at it or hold it in our hand. We must get the aspirin into our body to get its effect. Second, we must take enough into our body to get the effect. One or two 5-grain tablets usually suffice.

Few people, however, would expect a tenth or a hundreth of an aspirin tablet to cure a headache and it is not likely that it would. In other words, the therapeutic effect of a drug disappears with diminishing dose just as toxicity disappears with diminishing dose. As a matter of fact, aspirin has both toxic and therapeutic effects. About 150 5-grain tablets would kill the average man, and we count on the fact that this lethal effect has disappeared when we take one or two tablets for the therapeutic effect.

If it were not true that the human body can cope with sufficiently small doses of toxic chemicals, of necessity, the human race would have perished long ago; since almost all food and water, as well as the air we breathe, contain and have contained small amounts of materials which, in larger doses, would produce definite toxic effects.

The important problem with respect to the organic contaminants of water is whether they are in the range of concentrations that the body can cope with, or whether they are in the range which would produce minimal and possibly cumulative toxic effects.

Organic Contaminants in Food

The problem, as I have mentioned before, is analogous to the problem of incidental or intentional addition of organic materials, again including pesticides and surfactants, to our food supply. In the latter instance the U. S. Food and Drug Administration has been given authority to promulgate regulations establishing safe levels in food for the various additives. Only one class of additives has been singled out for special treatment and that is the class of chemicals which are found to induce cancer when ingested by man or animal. These latter, by law, are not to be permitted in food in any concentration other than zero.

Even with the carcinogens there is substantial agreement among experts that no-effect levels of ingestion exist, but there is substantial disagreement about our ability to select safe levels for human ingestion of most of them. As long as the experts cannot agree on safe levels of ingestion for human beings of materials found to induce cancer when ingested - at some level - by man or animal, their complete exclusion is in order; but I personally feel that when the experts both within and outside the Government who are best qualified to judge do agree that a safe level of intake for any of them can be assigned, the law should permit a tolerance to be established.

Thus, physiological criteria can often be used to detect effect levels of ingestion of a chemical. In our own Laboratory (4) we have for years been observing in chronic toxicity studies the effect of all sorts of chemicals on the blood pressure of dogs. We have frequently observed statistically significant changes in blood pressure in advance of any other criteria of effect and have then obtained an early clue to effect levels. We recognize that these changes in blood pressure are non-specific but they are, nevertheless, useful. We have also used observations of spontaneous activity of small animals in activity cages, the ability of small animals to maintain their balance on a slowly revolving roller, the work performance of dogs on a treadmill, and modification of reflex responses as physiological criteria of effect. Impending renal damage can often be recognized at an early stage as an impairment of the ability of the animals to produce a normally concentrated urine. And one might mention that recent Russian literature contains frequent references to the effect of chemicals on the Pavlov conditioned reflex as a criterion of toxicity.

While physiological criteria of effect are important as tools of toxicological investigation, it is unlikely that we would observe any immediate physiological effects in man from the ingestion of treated waters because the level of contamination would ordinarily be well below the effective dose level.

All of these methods apply to the evaluation of toxicity for mammals, since I take it that we are chiefly interested in the physiological effects of organic contaminants of water on man or his domestic animals. When we are dealing with water sources, however, we have an additional tool for the evaluation of safety and that is the biological survey, or study of the aquatic life in its natural environment. Contaminants, if present in sufficient concentration, may upset the delicate balance of aquatic life and thus reveal their presence and give warning of the necessity for a closer look at mammalian toxicity.

To sum up, water contains organic as well as inorganic contaminants, old and new. We have learned to deal effectively with the old contaminants but the increased and increasing use of new organic materials for various technological purposes raises the problem of their ultimate return to natural water sources and their appearance in small but finite amounts in public water supplies.

Establishing Safe Levels of Ingestion

On principle, if we can establish safe levels of ingestion for organic contaminants of food, we can establish them for organic contaminants of water or air. The methods applicable to one problem are also applicable to the others. What are these methods?

The most widely accepted method in the United States is to establish through feeding tests with animals a no-effect level of ingestion over two years, the practical life span of one species - the rat -, and over one or two years of another species - usually the dog. One assumes that the no-effect level for man will be a fraction - usually 1/100 - of the no-effect level for the test animals. While this method is time consuming and expensive, it has served well from a practical point of view in that judgments based thereon have in a number of cases stood the test of time and experience. There have been no conspicuous failures of this method.

Generally, we mean by a no-effect level of ingestion for animals that there have been no effects on growth rate, life span, or reproduction; no detectable biochemical or hematological changes; and no evidence of gross or microscopic pathological change attributable to the test material.

Another method for establishing safe levels of ingestion is the biochemical, or metabolic, approach. It can sometimes be demonstrated that a material cannot be absorbed from the gastrointestinal tract, or that if absorbed, it is converted in the body to known and harmless materials. So long as the level of ingestion does not exceed the body's capacity to deal with the material, it is safe. This method, while not necessarily less expensive than the 2-year feeding test, is usually less time consuming. Its applicability, however, is limited.

Chronic Toxicity Criteria

Since this Conference is concerned with the physiological aspects of water quality, it is appropriate to mention physiological criteria of effect of chemicals. A chemical will very often produce a functional effect at a lower dose level than an organic effect or, in a chronic toxicity experiment, may produce a functional effect before an organic lesion can be detected.

Evaluating Organic Water Contaminants

The problem of evaluating the safety of organic contaminants of water can be attacked by the same methods as are used for evaluating the safety of intentional and incidental food additives. It should be possible to determine safe concentrations for most organic contaminants of water and we should expect these safe concentrations to be finite rather than zero. The second problem from the public health point of view, is to make sure that the actual concentrations of organic contaminants in the water supply are below the maximum safe levels and in the range where no undesirable odor, color or taste is imparted to the water.

This second problem may be more difficult - or less difficult - to solve than the evaluation of safety. I am not a water engineer, so I do not know. I suppose that the two approaches to its solution would be: (a) to prevent to the extent practicable substantial amounts of organic contaminants from reaching natural water sources; and (b) to reduce the amounts of such contaminants which do reach natural water sources to safe and acceptable levels by the customary methods of water treatment.

It would appear, then, that we have the tools required for the evaluation of the safety of organic contaminants of water and we know the means for reducing these contaminants to safe and acceptable levels. If this knowledge is applied to the solution of the problem of safe and acceptable water supplies, it should be possible to avoid even minimal physiological or toxicological effects.

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THE CHRONIC TOXICITY OF DODECYLBENZENE SODIUM SULFONATE

By

Orville E. Paynter

The total tonnage of synthetic detergents produced in 1958 has been estimated to be over two billion pounds (1). The possibility that traces of these compounds might find their way into drinking water has resulted in attention being directed to this problem by the American Water Works Association and the Ohio River Valley Water Sanitation Commission in the United States, and by the Ministry of Housing and Local Government in Britain.

Because over half the yearly production of synthetic detergents is composed of a single commercial product, usually identified as dodecylbenzene sodium sulfonate but often referred to as alkyl benzene sulfonate (ABS), most of the interest and research has centered on this compound. Analytical methods for this class of compound in various types of water have been developed (2), and monitoring programs have been established for several river systems.

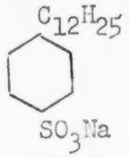
The results of the monitoring programs here and in Britain are in close agreement and indicate that the concentration of dodecylbenzene sodium sulfonate in raw water and in drinking water is substantially less than one part per million (ppm). Weaver and Coughlin (3) recently reported that the weekly average, for a five-year period, of surface-active agents in the Ohio River at Cincinnati was 0.16 ppm, measured as "apparent ABS" by the methylene blue procedure.

Even at this low concentration, the question of the safety of this water as a source of drinking water has some importance. Published reports indicate that dodecylbenzene sodium sulfonate, when administered to man and other animals for short periods of time, causes no observable ill effects. Until recently no observations of the long-term or chronic exposure effects of this compound had been reported.

Studies of Chronic Toxicity

The studies which form the basis of this summary have been published in detail in Toxicology and Applied Pharmacology (4, 5) and were undertaken to determine whether the long-term ingestion of dodecylbenzene sodium sulfonate in amounts exceeding those present in environmental sources would constitute a hazard.

Although the commercial product is a mixture of isomers and hemologues, the conditions of manufacture are very carefully controlled in order to give a highly reproducible product which may be identified structurally as:



The sulfonate group is predominantly, though not entirely para to the alkyl chain. The alkyl radical is predominantly 12 carbon atoms (90% C_{11} to C_{13}). The samples used in our studies were representative of good manufacturing practices and, therefore, can be considered a representative of the present commercial product.

Methodology of Animal Studies

Four hundred forty (440) weanling male and female albino rats were used in three two-year studies. One group of 240 rats received dodecylbenzene sodium sulfonate at dietary levels of 0, 1000, and 5000 ppm, and a second group of 120 rats received dietary levels of 0, 200, 1000, and 2000 ppm. A third group of 80 rats received the compound in their drinking water at a level, adjusted from time to time, to maintain a daily intake approximately the same as that of the rats receiving the 1000 ppm dietary level. Control rats for this third group received tap water.

All animals were individually housed. Fresh diets were prepared weekly, and fresh experimental water was offered three times per week. Animals, chosen at random, were sacrificed at 26 and 52 weeks and all survivors at the end of 104 weeks. Complete blood counts, blood urea nitrogen and hemoglobin determinations, icterus indices, and urine analyses were conducted on all sacrificed rats. Autopsies were performed on all animals that died or were sacrificed. During the autopsies the weights of the liver, kidneys, testes, and ceca (full and empty) of each rat were recorded. Tissue sections from 12 organs from each of 52 rats were prepared for microscopic study.

Reproduction, through two filial generations, was followed among representative rats from the 0, 1000, and 5000 ppm feeding groups. Criteria evaluated were fertility, lactation, litter size, survival, and growth, as well as hematological and pathological studies.

Results of Feeding Studies

The appearance and behavior of the test rats receiving the dietary levels of dodecylbenzene sodium sulfonate were comparable to the control rats throughout the study and no gross signs of toxicity were noted in any of the test animals. No statistically significant differences ($p = 0.05$) between the experimental and control groups were noted for growth, mortality, and food consumption.

Results of hematological and biochemical studies performed on representative rats during the course of the study showed no differences between the control and test groups. There were no gross pathological lesions found in any of the rats fed diets containing dodecylbenzene sodium sulfonate which could be attributed to the ingestion of the compound. Deaths that occurred among the control and test rats appeared to be due primarily to pulmonary infection. Microscopic examination of tissues taken from rats in the control and each test group showed no differences attributable to the treatment.

In one feeding study the organ/body weight ratios showed a statististically significant difference in the case of livers from females receiving the 5000 ppm dietary level. This difference, however, occurred only at the 26-week interval. At the two other intervals, 52 and 104 weeks, there were no differences observed, and at no time did the absolute liver weights show differences from the control. The liver/body weight ratios from the second study showed no significant differences for the livers. Because of this and the absence of any histopathological changes in the livers of any of the experimental animals, it is felt that the difference appearing in the 26-week 5000 ppm females is of no biological significance.

The cecum/body weight ratios (empty and full) present a somewhat confusing picture in that a significant difference was found in the data for the empty ceca of females at 26 weeks at the 1000 ppm level, but not at the 52- and 104-week intervals. In the data for the full ceca of males a significant difference was encountered at both low and high levels at 104 weeks. The ceca of the test rats presented no abnormal findings on gross or microscopic examination.

In the absence of any pathology related to the ingestion of dodecylbenzene sodium sulfonate or of significant differences in absolute weights of these organs, the variations in organ/body weight ratios could not be considered of biological significance.

Results of Reproduction Studies

The data from the reproduction studies revealed that dodecylbenzene sodium sulfonate had no adverse effect on fertility, litter size, lactation, or survival of the offspring. The results of the hematological studies, urine analyses, and blood urea nitrogen determinations performed on the first and second generation test rats were comparable to the results of similar studies performed on the first and second generation controls. Gross and microscopic examinations performed on the second generation test and control animals revealed no significant findings attributable to feeding of the test compound.

Administration of ABS had no significant effect on mortality, food consumption, or final body weight. A significant increase was observed in water consumption in the experimental groups with no accompanying evidence of stress. No departures from normal were encountered in the hematological and biochemical studies conducted on representative rats at termination of the two-year study.

Neither gross nor microscopic examination of tissues from rats that died or were sacrificed at termination of the study showed any evidence of pathology attributable to the ABS in the organs examined.

No statistically significant differences were observed between organ weights of experimental and control groups.

While statistically significant differences in liver/body weight ratios were observed in the male rats, there is nothing in the blood chemistry or organ pathology to suggest any evidence of stress in this organ. In the light of the negative pathology, the observed cecum/body weight differences in females are not considered to be evidence of tissue damage or toxicity.

Conclusions

Lifetime administration of dodecylbenzene sodium sulfonate at relatively high levels to rats by two routes, diet and drinking water, produced no evidence of toxicity as judged by the criteria evaluated. The levels of dodecylbenzene sodium sulfonate administered to the animals in these studies are made more meaningful by the following results of drinking water surveys made by the Soap Association's Research Steering Committee:

	Average "Apparent" ABS Content (ppm)	
	Series A	Series B
Well supplies	0.053	0.015
Lake supplies	0.02	0.015
Sewage-free sources	0.003	0.015
Mississippi River	0.02	0.038
Missouri River	0.038	0.014
Schuylkill River	0.08	0.04

As can be seen from this tabulation, even if all of the "apparent" ABS is true ABS, the amounts of dodecylbenzene sodium sulfonate present in drinking water are very small. To the extent that animal tests provide a basis for the assay of toxicity to humans, it would appear that these investigations assure considerable amounts of dodecylbenzene sodium sulfonate (much in excess of the amounts that might find their way into drinking water) could be consumed over long periods without harm.

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CANCER HAZARDS FROM NATURAL & ARTIFICIAL WATER POLLUTANTS

By

W. C. Hueper

Adequate and reliable epidemiologic, medical and experimental evidence attests to the existence of definite correlations between the contamination of the human environment with certain specific physical and chemical agents and the occurrence, frequency and local distribution of cancers in man residing in different areas, engaged in various occupations and exposed to numerous consumer goods containing such carcinogenic agents.

While the type and amount of information available in regard to occupational and environmental carcinogenic air pollutants and their effects upon man have obtained a level permitting their practical application for the control of human cancer hazards, the data available on cancer hazards from pollutants of the soil and of the water are still rather rudimentary and in urgent need of comprehensive elaboration and expansion for permitting their use for preventive purposes.

I. The Problem

Although water, like air, is one of the most important agents essential to the maintenance of all forms of life, very little definite information is on hand concerning the existence and types of actual and potential cancer hazards to man which may originate from the presence of recognized or potential carcinogens in water. Carcinogenic substances may be introduced into public water supplies not only with the discharge of urban and domestic sewage and the release of industrial wastes, but also by the pollution of ground and surface waters with atmospheric carcinogenic contaminants present in rain water, or with carcinogens introduced into it by mechanical or physical means from natural or industrial substances attached to the vegetation or incorporated into the soil and leached into the ground water. In fact, investigations of Stocks and Davies in England, Legon in Wales and Diehl and Tromp in Holland have revealed the existence of statistical correlations between the character of the soil and the gastric cancer rate of different localities.

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The carcinogenic agents which may be and are being introduced into sources of public drinking water supplies from such sources range from various inorganic and organic chemicals to radioactive materials and possibly even viruses, such as perhaps the hepatitis virus. It is obvious that with the rapidly increasing urbanization and industralization of the country and the greatly increased demand placed on the present resources of water from lakes, rivers and underground reservoirs, the danger of cancer hazards from the consumption of contaminated drinking water will grow considerably within the foreseeable future.

A critical appraisal of the present status and prospective development of cancer hazards created by water pollutants, therefore, appears timely and needed so that not merely the appearance of isolated cases of cancers caused by such contacts may be prevented but that especially also the occurrence of local and general epidemics of cancers of such a causation may effectively be forestalled by the introduction of rational technologic measures aimed at the maintenance of a safe and adequate drinking water supply for the general population.

II. The Evidence

1. General Observations

The immediate need for such studies is suggested by recent observations of Diels and Tromp from Holland, who found, that cancer death rates in municipalities with a water system since 1915 tended to be lower than in those without a water system, and that municipalities receiving their drinking water from rivers had a higher cancer death rate (606 per 100,000) than those which obtained their drinking water from bodies of water in heaths (595), dunes (585) or wells (568).

An additional indication for such investigations is an observation of Wedgwood and Cooper who studied sludge from sewage for polynuclear aromatic hydrocarbons in England and demonstrated among various chemicals of this type also two carcinogens, namely 2,4-benzpyrene and 1,2-benzanthracene. They suspected that these chemicals might have been introduced into the sewage with effluents discharged from gas works, washings from macadam roads and atmospheric soot washed from the air by rain.

The recent investigations of the National Water Quality Network in this country revealed marked differences in the content of aromatic chemicals in the water of American rivers and lakes. While the average content on aromatic chemicals ranged from 1 to 4 parts per billion, it stood at 11 to 37 parts in the Mississippi river at Cape Girardeau, but was 1 part at Vicksburg. The aromatic chemical content of the Ohio river was from 4 to 48 parts at Cincinnati, and 3 to 11 parts at East Liverpool, while the Hudson river at Poughkeepsie contained 3 to 80 parts of aromatic chemicals per billion. Such variations in the aromatic chemical contents of the water of the various rivers at different sites and at different times reflect, in part, the types and quantities of industrial and domestic effluents released into these bodies of water in different areas; in part, they are the result of fluctuations in the amount of water and the speed of flow present in these rivers at different periods.

If, therefore, cancer hazards should be related to the consumption of drinking water obtained from bodies of water thus polluted, one should anticipate that the carcinogenic effects produced in the exposed populations should not only differ in degree i.e., the number of individuals affected but probably also in type, i.e., in the organs involved in different localities and should depend on the particular source of drinking water used.

2. The Facts

a. Arsenic. The classical and so far unique example of carcinogenesis in man associated with and resulting from the consumption of drinking water containing a known human carcinogen is presented by the occurrence of cancers of the skin and possibly also of the liver in members of two population groups, one residing in the district of Reichenstein, Silesia, and the other living in Cordoba Province, Argentina. The former endemic occurrence of cancers among the people in Reichenstein was related to the presence of arsenic in the drinking water leached into the ground water from arsenic containing slag heaps of the gold and silver mines located in the vicinity (Kathe). The inhabitants of this area suffered for many years from chronic arsenic poisoning, which in some cases became complicated by cancerous developments in the skin and the liver. Following the construction of a new water supply in 1928, this interesting source of endemic arsenicism and arsenic cancer has become a matter of historical interest (Hueper).

Recent reports from Argentina indicate that similar conditions exist in Cordoba Province where in certain parts drinking water is obtained from rock formations containing large amounts of arsenic (Arguello; Tello). In addition to a frequent occurrence of chronic arsenicism, the inhabitants of this region suffer also from a high incidence of cancer of the skin developing on the basis of a chronic arsenic dermatosis and exhibiting a topographical distribution characteristic for arsenic cancer.

Because of these observations it may be pointed out that similar contaminations of public waters and of the water supply of communities with arsenic leached from slag heaps of copper ore smelters and originating from arsenic containing smelter fumes may exist in several areas of the Rocky Mountains (Salt Lake Valley, Utah; Deer Lodge County, Montana). Such conditions may also be created in areas where orchards, forests and fields have been dusted or sprayed for many years with arsenic containing pesticides, and where thereby a serious contamination of the soil with arsenicals has been produced (Northwestern States).

The potential carcinogenic importance of such exposures depends on the fact that such exposures to arsenic contained in drinking water contribute to the general dietary exposure of the population from arsenical pesticide residues and from arsenicals used as feed additives in the production of food animals, such as chickens and cattle, present in foodstuffs and from the still rather widespread use of arsenical insecticides and herbicides in the home environment.

b. Radioactive Chemicals. A second, but less conclusive illustration of endemic carcinogenesis presumably results from the consumption of drinking water or bathing in water contaminated by another carcinogenic agent, i.e., radioactive chemicals. A survey of the rock formations for radioactivity in those parts of the Erzgebirge, in which the radioactive mines of Schneeberg and Joachimsthal are located, showed that the granite contained 4.57×10^{-12} gm. of radium per gram of rock. The water of the entire region is radioactive, many springs containing 50 Mache units per liter. In Oberschlema where the water is especially rich in radioactive material because of the disintegration of pitch blende present in the rocks, the water may contain up to 12,000 Mache units or 1/4,000 mgm. of radium per liter. The average radioactivity of the water in creeks and rivers, however, is much lower ranging from 5 to 12 Mache units. Radioactivity is also demonstrable in the soil, plants, animals and man living in these regions.

In following up these physical findings, Lange prepared a geological map of this region, marking the radioactive and non-radioactive districts. When he traced the local origin of the 266 proven cancer cases, observed in the surgical department of the regional hospital, he found that the general cancer incidence of the radioactive regions to be three-fold that of the non-radioactive regions, which contained mainly phyllites and glimmer shale.

The widespread natural radioactivity of waters, springs, ponds and creeks in several parts of the United States, such as Arkansas and the Colorado Plateau has been accentuated during recent years in these and other parts of the United States by the release of radioactive wastes from uranium ore smelters and refineries and from atomic energy plants and similar establishments. This development may deserve, on account of the cited observations, serious and intense interest from all parties concerned so as to avoid the future epidemic occurrence of radioactive cancers among the population of regions obtaining their drinking water and food supply from areas in which the raw water contains hazardous amounts of radioactive material (Animas River, Durango, and Colorado River, Rifle, Colorado) (Love and Thatcher; Toffler). The possibility of dangerous concentrations of radioactive materials in rivers and creeks near such establishments is especially marked during the dry season (National Water Quality Network, Suppl. I. and Ann. Compil. 1957-1958).

The rapidly growing use of radioisotopes in medical and research laboratories and in industry is bound to add to the pollution of water supplies with radioactive materials of various kinds and, therefore, deserves attention as a possible contributory source of cancer hazards to the general population.

In addition to a direct radioactive effect of such water pollutants on consumers, attention should also be paid to direct and indirect radiation effects by radioactive matter on other chemical constituents, especially those of industrial origin which may simultaneously contaminate the water. Such effects may be associated with chemical changes elicited by the production of peroxides or may be related to intramolecular rearrangements of atoms and groups of atoms, such as polymerization, depolymerization and crosslinkage formation, occurring under the impact of ionizing radiations. An exploratory study into the chemical end products of such possible interactions between radioactive and non-radioactive water pollutants seems to be indicated for assessing properly the entire scope of potential cancer hazards for consumers of drinking water containing radioactive pollutants.

A periodic survey of fish and other marine animals, including mammals living in such bodies of water for evidence of cancerous growths and malformations may perhaps yield information on the existence of cancer hazards which would precede by many years any evidence obtainable in man. Several years ago the Bureau of Fisheries and Wildlife, U. S. Department of the Interior, has been alerted to the usefulness of such investigations and has remained in contact with the Environmental Cancer Section, NCI in such matters. While so far no evidence has become available on this particular aspect of environmental cancer genesis in fish, several observations have been made and will be mentioned at the proper place where cancers in fish or the demonstration of carcinogens in the tissues of marine animals have suggested the action of carcinogenic pollutants in the production of cancers.

The occurrence of an osteogenic sarcoma in a muskrat living in the vicinity of an atom energy plant and feeding on vegetation which had taken up radioactive strontium from polluted water has demonstrated the reality of such reactions in animals and has thereby suggested the possibility of similar cancerous complication in man, unless proper and adequate preventive measures are being taken. Apart from such a direct carcinogenic effect of radioactive water pollutants, radioactive matter ingested with drinking water increases the total radioactive body burden sustained from other sources including contaminated foodstuffs and air pollutants and thereby may contribute to the development of radiation cancers in the general population.

3. The Probabilities

Apart from these two types of water pollution with natural and industrial carcinogens which definitely or presumably resulted in the development of cancers among individuals using such waters for drinking purposes, there have been demonstrated instances of industrial water pollution which were followed by the accumulation of industry-related carcinogens in marine animals or which released carcinogens into the water capable of producing cancers in mice when applied in the form of eluants from water adsorbates. Since any evidence concerning a carcinogenic action on consumers of such waters, on the other hand, is at present lacking, these pollutants can only be listed as probable sources of human carcinogenesis.

a. Petroleum and Petroleum Products. Petroleum refinery wastes have been identified in surface waters into which such industrial plants discharged their wastes (Rosen and Middleton; Ruchhoft, Middleton, Braus and Rosen; Middleton and Rosen). Wedgwood and Cooper reported the recovery of various polycyclic aromatic hydrocarbons from industrial sludge and sewage including the carcinogen, 3, 4-benzprene. Fuel oils, lubricating oils and cutting oils are being introduced into lakes and rivers from garages, service stations, petrochemical plants, metal working plants and ships, and may appear as films or scum on the surface of the water or may be suspended in it as an emulsion. Such oily pollutants may interfere with the filtration of such raw waters, for drinking purposes. Their continued presence in the drinking water is indicated by spectrophotometric observations made on such waters especially whenever such waters are being used without any previous filtration for home use.

Several years ago Ruchhoft from the Sanitary Engineering Center, Public Health Service, prepared adsorbates of several raw and finished waters by filtering large amounts of water through filters of activated carbon. The eluants of these adsorbates were tested in mice by repeated cutaneous application. Of the several eluants examined, those which were obtained from effluents of an oil refinery with a catalytic cracking operation as well as from the water of a canal into which these effluents were emptied, produced cancers of the skin of mice. These findings indicate that carcinogenic chemicals were being released with the refinery effluents into the body of water serving as a source of drinking water for a large metropolitan area (Hueper and Ruchhoft). It is also of interest that Kotin et al., observed the occurrence of cancers of the mouth among fish feeding in the mud of waters contaminated with effluents from nearby oil refineries at Los Angeles Bay and that Shimkin et al., as well as Cahnmann and Kuratsune, demonstrated the presence of the carcinogen, 3,4-benzpyrene, in extracts of barnacles and oysters, respectively, living in waters contaminated with carcinogenic ship fuel oil.

Considering the fact that petroleum tars, tar oils, cutting oils, crude paraffin oils, fuel oils, and asphalt have been shown to be carcinogenic to the skin of man and mice, the pollution of waters which may either serve as sources of drinking water supply or which may furnish food animals contaminated with carcinogenic chemicals contained originally in petroleum products should be of distinct importance from a public health viewpoint.

Mention may be made also of the observation that in certain rural parts of Texas, the drinking water is contaminated with crude oil which seeps into wells. No information is available at present whether or not such pollution may create a cancer hazard for the population of such regions.

A contamination of public waters may also result from the use of kerosene, methylated naphthalenes and similar petroleum products employed as vehicles of insecticide sprays distributed from airplanes or directly placed on the surface of the water for the killing of mosquitoes or entering bodies of water with the rain water from oiled, tarred or asphalted roads or with the effluents of industrial plants, automobile service stations, garages, etc., using petroleum oils and greases.

Any exposure to carcinogenic petroleum chemicals contained in the drinking water which the general public might sustain would be in addition to alimentary contacts to similar chemicals contained in and contaminating foodstuffs, when insufficiently refined petroleum waxes and mineral oils are employed in the manufacture of wrapping and packaging materials, in the processing of foodstuffs and for medicinal purposes (Hueper; Falk, Kotin and Miller; Schmahl and Reiter).

b. Coal Tar and Tar Oils. A pollution of public waters with similar carcinogenic chemicals results through the release o effluents from gas plants, coke oven operations, tar distilleries, tar paper plants, wood pickling plants, tarred roads, etc., since coal tar, pitch, creosote, and anthracene oil are known human carcinogens. It is, however, undetermined whether or not an ingestion of such products of the distillation of coal will cause cancers of the alimentary tract in man, although such an action has been suspected and is likely. No epidemiologic information is available whether populations obtaining their drinking water from bodies of water into which such effluents were released have any excessive liability to cancers. Since in at least one large Midwestern metropolitan area unfiltered lake water containing effluents from nearby located steel plants and oil refineries and often distinctly flavored by such products is supplied for public consumption, such pollutants may not be without carcinogenic significance to the local cancer panorama and, therefore, are of distinct public health interest.

Experiments with adsorbates on activated carbon obtained from the water of a river near the entrance of effluents from a large coke oven plant gave negative results, when the eluants were painted on the skin of mice.

Druckrey and Schmaehl recently called attention to the fact that drinking water might be contaminated with carcinogenic aromatic hydrocarbons since the pitch used for the coating of water pipes is carcinogenic to animals. It is not likely, however, that the degree of any potential cancer hazard which might be related to this source can be compared with that which might result from the contamination of drinking water by similar chemical carcinogens from industrial wastes.

c. Chromium. The carcinogenic properties of certain chromium compounds encountered occupationally, mainly in chromate producing plants and pigment operations, are well established by the demonstration of a high incidence rate of lung cancers among the workers employed in such establishments and by the production of sarcomas and carcinomas of the soft tissues and of the lung in rats and mice receiving implants of such compounds. Because of the high carcinogenic potency of some of these compounds, it is of importance that an excessively high chromium content of drinking water, particularly water in wells, has been demonstrated in several communities located near several plants using such compounds and releasing them in wastes into the air and sewage thereby contaminating the ground water.

While it has not been established that the ingestion of water contaminated with chromium creates a cancer hazard for its consumers, this problem is of distinct general importance, because such exposures occur not only as the result of an industrial pollution of public waters, but to a lesser degree also from the use of stainless steel utensils in the preparation of food. The population groups having contact with the more massive doses in industrially contaminated water, therefore, may serve as a test group for ascertaining whether or not a prolonged ingestion of chromium may exert a carcinogenic effect and for obtaining information on the concentrations of chromium which may be dangerous in this respect, since chromium occurs as a trace element in most living matter, including man.

d. Aromatic Amino- and Nitro-Compounds. The high carcinogenic potency of certain aromatic amino-compounds, such as beta-naphthylamine, benzidine and 4-aminodiphenyl, for man is well established by comprehensive experiences made in the dye and rubber industries. Since these chemicals as well as carcinogenic nitro-analogues used in the production of the amino-compounds and of several carcinogenic dyes (aminoazodyes, amino-stilbenes, tri- and diphenylmethane dyes, phenetidine derivatives, etc.) may be released into public waters with wastes of dye- and rubber manufacturing establishments of pharmaceutical factories, textile dyeing plants, plastic production operations and similar industries, it is important that drinking water supplies polluted with effluents of such plants studied for the presence of these chemicals. Some of the intermediates, such as chloronitrobenzol, demonstrated in the water of the Mississippi river, have exhibited a remarkable degree of stability (Middleton et al.). Chloronitrobenzol discharged into the Mississippi at St. Louis was still demonstrable in the water obtained at New Orleans.

4. Potentialities.

In addition to the various water pollutants representing known or strongly suspected human carcinogens, there are others which have been shown to elicit cancers in experimental animals and which, therefore, must be considered to be potential human carcinogens. Prominent members of such water pollutants are several pesticides and surfactants, which during recent years are being introduced in rapidly increasing amounts into public waters particularly with large-scale dusting and nebulizing operations of pesticides and herbicides, with effluents of pesticide producing plants and with domestic and industrial sewage, containing detergents, respectively.

a. Pesticides and Herbicides. Observations made on animals have shown that a number of pesticides, herbicides and preservatives, such as DDT, Aramite, carbontetrachloride, acetamide, thioacetamide, thiourea, thiouracil, aminotrizzole, several urethane derivatives, (isoprophylchlorophenylcarbamate) and beta-propiolactone, are capable of eliciting benign and/or malignant tumors in various organs. Their sites depend upon the chemical and the route of application (Hueper). Some of these chemicals when fed, produced hepatomas or hepato-or cholangiocarcinomas of the liver in mice/rats and/or dogs (DDT, Aramite, Carbon tetrachloride, acetamide, thioacetamide). Others caused the development of adenomas and carcinomas of the thyroid in rats (thiourea, thiouracil, aminotriazole). Carbamate derivatives have stimulated the precocious and accentuated appearance of pulmonary adenomas and carcinomas in the lungs of mice and rats and have displayed cocarcinogenic properties (isopropylchlorophenylcarbamate). Beta-propiolactone has been shown to be a potent carcinogen to the skin and subcutaneous tissue of mice and rats.

Although it is unlikely that the amounts of these chemicals polluting drinking water supplies will ever reach levels which are sufficient for causing any cancers by themselves, such pollutants nevertheless may create a certain hazard by aggravating the exposure to these and similarly acting environmental carcinogens to which the general population becomes exposed in the form of food additives and contaminants, ingredients of consumer goods, and environmental or occupational pollutants and agents. The recent epidemic occurrence of primary cancers of the liver among rainbow trout in fish hatcheries affecting from 40 to 80% of the fish and probably attributable to a feed ingredient, may serve as a warning that similar sequelae may be in store for human population groups, unless proper precautionary measures are being taken.

b. Surfactants. Similar considerations must be applied to the pollution of drinking water supplies with domestic and industrial surface active agents, especially detergents, which are extensively used for cleaning purposes in the household, car washes, restaurants, hotels, camps, offices, and industry, such as especially slaughter houses, canneries, vegetable and fruit processing plants, bottling establishments of soft drinks, milk, etc., for the degreasing of metal parts, pickling of steel, electroplating, textile plants, ore dressing plants, detergent factories and in dry cleaning establishments, as well as ingredients of lubricating oils and pesticide sprays.

Since the effluents from all these sources of detergent uses enter bodies of public waters, they may create at some places a considerable degree of pollution of drinking water supplies. Because of the great chemical stability of these agents and the inability of the ordinarily used and economically practicable methods of filtration of water intended for human consumption to withhold all detergents present in the raw water, these chemicals have been introduced into many urban water supplies at an increasing rate for a number of years (Hammerton, Middleton et al.).

Household detergents and many industrial detergents are being ingested either as pollutants of drinking water or as residues on kitchen utensils and dinnerware cleaned with detergent solution and not subsequently rinsed with water may create potential human cancer hazards by two different mechanisms (Love and Thatcher). Detergents when in contact with the mucosa of the alimentary tract may facilitate and accentuate the contact of the mucosa with carcinogens of various types ingested with the food and may simultaneously increase the permeability of the mucosa cells to these extraneous carcinogens, thereby activating or aggravating their carcinogenic effect upon these tissues or their transport and their action on remote tissues (Setala). Observations made with the experimental use of detergents administered by mouth in combination with polycyclic aromatic carcinogens to mice support the occurrence of this role of detergents in chemical carcinogenesis.

The other facet is related to the cocarcinogenic properties possessed either by the dodecylbenzol mixture itself from which by sulfonation the detergent dodecybenzolsulfonate present in all household detergents is produced or by an impurity, dodecane, sometimes present in this mixture (Shubik et al.). It is uncertain, however, whether the cocarcinogenic effect of these materials observed on the skin of mice when exposed to a known carcinogen, is present also on the alimentary mucosa and whether it exists for tissues of man. Nevertheless, the possibility of such an effect from household and industrial detergents upon the alimentary mucosa cannot be dismissed, especially since dodecylbenzol is a chemically indefinite mixture of alkylbenzol compounds obtained from petroleum, and because other surfactants used in the food industry have shown cocarcinogenic properties (Tween 60 and Tween 80) or have displayed even carcinogenic qualities (polyozyethylene (8) stearate) when fed to mice and rats, respectively (Fitzhugh et al.).

c. Miscellaneous Pollutants. Apart from the numerous unintentional water pollutants of mainly industrial origin for which any information concerning carcinogenic properties is totally lacking, there are several intentional additives, such as chlorine, fluorine, water softners, and water evaporation retardants which deserve consideration in this respect. The recently spreading use of drinking water medicated by the addition of fluorine compounds for the prevention of tooth decay has given rise to the claim that such practices might create a cancer hazard for the consumers affecting particularly the thyroid or the bone. At the present time no valid and scientifically acceptable evidence supports such an allegation concerning a carcinogenic action of fluoridated water.

For areas where water is in short supply such as our Southwestern States, it has been proposed to spread on the surface of water in lakes and reservoirs a monomolecular film of hexadecanol for retarding the evaporation of water. Before such practices should actually be adopted it would be most wise to conduct on animals long term experiments with chemicals intended for such a use for excluding the addition of possibly carcinogenic substances to sources of drinking water supply.

5. General Considerations and Control Measures.

It is apparent from the preceding discussion that the available knowledge on intentional and unintentional, actual, suspected and potential carcinogenic pollutants of water is highly defective not only concerning the chemical nature of such substances but also in regard to their carcinogenic effectiveness on the consumer. Even where known human carcinogens are demonstrable in water used for drinking purposes, there does not exist, with a few exceptions, any reliable and useful evidence which proves or suggests whether a long continued ingestion of such water containing rather minute amounts of the carcinogenic material might create an actual cancer hazard to members of the consuming population.

The worst that can at present be stated regarding a cancer hazard from specifically polluted water is that the prolonged or life-long consumption of such water will contribute to the total carcinogenic burden from all sources for the exposed individuals. The decision whether the contributory role of carcinogenic water pollutants to the causation of cancers of all sites or specific sites is a minor or a major one depends on the availability of a much greater amount of reliable chemical and epidemiologic data for exposed populations, than those on hand at present. It will be necessary that in the future public health and sanitation officials include in their work on the maintenance of a safe water supply not only considerations of microbiologic safety but also of chemical and radiologic safety.

Since some conclusive and a great deal of circumstantial evidence suggests that water pollution with industrial and industry-related agents might create not only toxic but also carcinogenic health hazards to the consuming public, it seems to be wise for public health agencies to consider in cooperation with industry the development and institution of effective and economically feasible control measures which may prevent or reduce such possibly very dangerous contaminations of a vitally important material and foodstuff. Such sanitary safety measures represent indeed a rather cheap price to pay for the prevention and control of health hazards created by chemicals and by-products and waste products of industry which has brought many benefits and advantages to the people of all countries.

OPEN DISCUSSION

DR. NELSON: I would like to request Dr. Gaufin to comment on a subject raised in the last paper. Dr. Gaufin was, I believe, involved in some of the earliest phases of the study of trout liver cancer.

DR. GAUFIN: I would like to ask questions of Dr. Hueper. Early in 1960, some trout were sent to me from Idaho as well as from the Logan Hatchery in Utah, and I was asked to examine them. At that time, it appeared as if there was a divided opinion as to whether or not they exhibited a condition of hepatoma of the liver or cancer.

DR. HUEPER: It was both hepatoma and hepatocarcinoma.

DR. GAUFIN: Furthermore, it appeared as if the condition were restricted to just certain hatcheries, whereas other hatcheries, using the same food, did not have it. Can you comment on that?

DR. HUEPER: A representative of the Bureau of Fisheries came to me with a report of this condition, and I was left under the impression that the feed supplied by only one manufacturer in Idaho appeared to be responsible for the occurrence of these cancers. They are real cancers, not hepatomas. In some of the fish they produced metastases, and also invaded the adjacent muscle tissue.

I requested that the Bureau investigate fish in all hatcheries, not only those in the West but also in the East. I was told at that time they had no evidence cancer of the liver occurred among rainbow trout in the East. In the occupational cancer field, impressions or say-so do not constitute good evidence. An investigation was started and, as the picture developed, we found that cancers of the liver were observed in almost all hatcheries; not in all, but in almost all hatcheries in Pennsylvania, West Virginia, and Maryland. My associate is presently in Virginia

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to find out whether the condition exists in Virginia hatcheries. There can be no doubt about it, this is a very widespread occurrence.

The second thing we found is that this condition is not of recent date. The first observations on the presence of primary cancer of the liver in rainbow trout were reported in 1955. That report escaped attention.

What is the cause of this condition, we don't know. We are running experiments with feed, extracts, and various ingredients. I hope we can find the answer for the benefit of the trout industry, because I think it is important. I have been informed that the law forbids the shipment of these trout, or of meat from any diseased animal across State lines. Since these fish are, to a large extent, used for eating purposes rather than for sport purposes, this condition has had a serious effect on the hatcheries.

DR. GAUFIN: Another question, do you know whether or not trout such as brown, brook and cutthroats are also affected?

DR. HUEPER: Dr. Payne is in Virginia to determine the answer to that question. He is visiting hatcheries in which brown trout and brook trout are raised, and we plan to find out whether this epidemic extends to other types of trout.

We have also already made arrangements with the Bureau of Fisheries to later study lake trout and salmon. Trout are a member of the salmon family, as you know, and we want to study the whole family.

DR. GAUFIN: Since this condition evidently affects only the liver of fish, and as far as I know no one eats the liver, is it safe to eat trout taken from streams and lakes?

DR. HUEPER: Let me ask you the question: After you have seen fish having cancers illustrated in the pictures I have shown, would you eat the meat?

DR. GAUFIN: Well, I have seen some fish in which there is just a mild infection. It doesn't seem to extend to the rest of the fish. In such cases, is there danger of transmission of cancer?

DR. HUEPER: We don't know. If I knew the cause, I could give you the answer. This is the only reply I can give, but for information, I will cite the experience in my own laboratory.

I brought back the affected fish and posted them in my own laboratory. That day, three assistants who are very fond of fish were present. After they had seen the tumors, I offered them one big ten pound trout which was normal. Their emphatic answer was "Oh, no, Doctor."

DR. HAYES: I would like to ask if any studies are being made of this condition from the standpoint of possible virus effects?

DR. HUEPER: Yes, we have raised that question, too. At the present time, we have no evidence that it is a virus disease. Histologically, the tumor has no inclusion bodies, which is no proof one way or the other. One of the main theories advanced is that the condition is genetic. This doesn't explain very much, since rainbow trout in the different hatcheries are far from being inbred.

DR. HAYES: Are studies being made of this disease by those who are qualified to study virus effects of cancer?

DR. HUEPER: I have been told, although I am not sure about it that the virus aspect is being studied in California, where a study was underway for several months before I became acquainted with it. I am advised they are working on the virus concept, but how much has been done I don't know. I am not directing this research; I am just helping.

DR. STOKINGER: Dr. Hueper, I have heard it expressed recently that we must be very careful in ascribing cancers to arsenic because the condition is related to a specific form of arsenic; namely, arsenite, as formerly in Fowler's solution. I would like your comment on this: Do you think all forms of arsenic are carcinogenic, or only a particular form?

DR. HUEPER: Well, we raised a question about arsenic as the origin of these trout carcinomas. Arsenic is a liver poison, and it produces cancers of the liver in man. Quite a number of cases are now on record among vineyard workers in Germany. These workers who used arsenical insecticides between 1920 and 1940 are now developing cancers of the skin - multiple cancers of the skin, cancers of the lung and cancers of the liver -- sometimes, all three types of cancers in one person.

Now, the fish are treated, with arsenic or sometimes get arsenical amebicides in their feed. We studied those arsenicels first; but when we inquired, we learned they are used only very occasionally. And it doesn't appear that this source is responsible for these cancers. In my opinion, one ingredient in ordinary food which isn't suspected at all must be responsible.

DR. MCKEE: I wish to ask Dr. Paynter at what concentration dodeclybenzene sodium sulfonate can be tasted in water and at what concentrations it will foam? In other words, let us digress temporarily from physiological effects and consider esthetic effects.

DR. PAYNTER: I must confess I know very little about water treatment. I have been able to taste -- or rather get a sensation - at the 2,000 ppm level. I am not a taster in a sense that many of my colleagues taste and smell things that I do not. I really can't answer your question as to the level at which the "normal" population can taste ABS.

I have been told, but do not know this for a fact, that if the level of dodecylbenzene sodium sulfonate in water exceeds about 1.0 ppm, foaming is apt to occur. I do not know if this is a correct figure.

DR. NELSON: Does anyone else want to comment on that point?

DR. WOODWARD: The ABS doesn't have much taste. We made studies of this at Cincinnati, and I think others have also. It causes a "slippery" feeling at a concentration of about 15 ppm. As far as taste or odor is concerned, the pure material has essentially none. Packaged products, of course, may contain perfumes and other things, so that is a different story.

The foaming concentration is rather uncertain, but it is probably at or below one part per million under some circumstances. It depends also on the water composition.

DR. HAYES: I would like to make an observation based on my own home. With the dishwashing as done by wives, husbands, and children, the amount of detergent we are likely to get from water is small compared with the amount that remains on washed dishes and utensils. Whatever the toxicity may be, I think that a major ingestion of detergent comes from direct contamination of vessels.

DR. WOODWARD: That also has been investigated, and your impression isn't quite right. The amount of detergent that remains on dishes is rather negligible, if the rinsing operation is reasonably decent.

DR. HAYES: We now have a dishwashing machine. Maybe it does a better job than people.

DR. GRIFFITH: A study of residues on dishes is included in the report of the British Government's Committee on Synthetic Detergents. I believe cups were washed in normal concentrations of household detergents, drained dry without rinsing, and refilled with distilled water. Subsequent analysis of the distilled water showed levels of about .2 ppm of ABS.

DR. NELSON: I have a question to ask Dr. Zapp. It seems to me that a fairly large proportion of industrial chemicals reaching the market are tested, at least to some degree, for toxicity. These are not, of course, the ones that commonly find their way as contaminants into streams. In other words, chemicals that are saleable are tested. But to what extent have other materials from industrial processes, such as debris, by-products, and so on, been examined for toxicity in a routine manner?

DR. ZAPP: Well, the principal problem from an industrial point of view regarding industrial wastes seems to be the effect they will have on aquatic life rather than on human life. In other words, if a manufacturing plant discharges wastes into a stream, and these upsets the biological balance of that stream - result in the death of game fish and things of that story - of course, industry is in trouble. Industry is, I think, well aware of this problem. We have a representative of the DuPont group who is responsible for looking into these aspects of company plant wastes, particularly of new plants.

It always is good policy to make a survey of the biology of a river or any body of water into which waste is to be discharged. Of course, every possible effort is made to keep the contamination of that water to a minimum. Then, the original biological survey is followed up with repeated surveys to make sure the balance of nature has not been disturbed. In addition to considering potential effect on aquatic life, industry does consider the possible harmful effects of its plant wastes on man and other mammals, but in most cases aquatic life is the most susceptible.

MR. ONGERTH: My question bears no relationship to a specific chemical: Have animal feeding studies been carried on for multiple generations at the level of ten to twenty generations? If so, what are the relationships between two-year feeding studies and these longer periods?

DR. NELSON: Would you like to respond to this question Dr. Fitzhugh?

DR. FITZHUGH: It is a hard question to answer. I don't have any direct relations with any experiments which I can refer to directly involving ten or so generations. Personally, I know of experiments which have gone three generations, and that is about the maximum. In some instances, they correspond to the two-year test, and sometimes they don't. Ordinarily, a two-year test of chronic toxicity will bring out toxic effects similar to those shown by the three generations test. More than three generations of testing may be an advantage, but this is as far as I have gone in my testing procedure.

MR. ONGERTH: There doesn't seem to be much difference between two and three. Suppose you have another order of magnitude.

DR. FITZHUGH: I can't answer that because I have not used a greater number of generations.

DR. MONTY: Nutritional experiments involving successive generations are subject to technical problems which restrict the number of generations through which the experiment remains useful. Problems concerned with maintenance of constant and adequate dietary conditions, control of infectious diseases, and soforth, become grossly magnified when experiments span a number of years. The studies of multiple successive generations add a new problem to the list--that of the possibility of accidental genetic divergence between the experimental and control groups. I am familiar with only a handful of attempts at multiple generation nutritional experiments with animals. In each of these cases, it was difficult or impossible to interpret, or assign significance to, differences encountered after about the third generation. I guess we haven't learned as yet how to do such long term experiments.

MR. ONGERTH: What is the risk, then, in drawing our conclusions on two generations?

DR. MONTY: Indeterminable.

MR. ONGERTH: How much value are these animal feeding studies, then?

DR. MONTY: Well, they are certainly better than no studies.

DR. ZAPP: I think you can say more than that. We have had definite experience with a number of chemicals which have been included in our diet for many, many generations, in relation to which this accumulative effect we are worrying about has not occurred. This experience would be true even of some of the natural carcinogens.

DR. HUEPER: And in related malformations in the offspring.

DR. NELSON: Do we have any further comments?

DR. RYCKMAN: I would like to address a question to Dr. Paynter. I have wondered if you checked the results of your studies with reports in the literature on the feeding of alkyl benzene sulfonate to hogs and chickens? Have you made any investigation at all as to the effect of these materials on fat metabolism? I infer you were hinting at this a little in your paper when you indicated that there was no observed differential effect on the rate of growth of the animals used as controls with those that were fed alkyl benzene sulfonate.

DR. PAYNTER: What we really were looking for were adverse effects of ABS. We know that many surface active agents have a definite beneficial effect on the growth rate, especially in young animals. Dodecylbenzene has. ABS has been fed to hogs, and there is some indication that it does add weight to the animal.

DR. RYCKMAN: At what levels?

DR. PAYNTER: I am not clear on this, and I might say that study of the beneficial factor requires a large number of animals and good statistics. Whether or not it is real, I don't know. It is not commercially practical to feed hogs on this material.

While I have been particularly interested in the adverse effects, many surface active agents can be used for their benefical effects; some of them at quite low levels, less than a part per million.

DR. RYCKMAN: Just one question. Do you regard the increase in fat metabolism as a benefical effect?

DR. PAYNTER: Under certain circumstances, yes.

DR. GRIFFITH: In response to Dr. Ryckman's first question, there is a review in the June 1960, Journal of the American Water Works Association which mentions a number of nutritional studies of ABS carried out with domestic animals. Growth of weanling pigs has been stimulated over a short period by .2 percent of ABS in the diet. However, work has been done with hogs in Germany in which similar levels of ABS have been fed for several months with no stimulation in growth.

DR. HUEPER: I would like to ask Dr. Paynter, since there have been carcinogenic studies of dodecylbenzene sodium sulfonate reported by two different investigators whether he has made studies of this type; or if he can report any observations on any neoplastic manifestations.

DR. PAYNTER: To answer your question directly, no. We have not conducted studies designed specifically for carcinogenic effect. However, in all of our two-year studies, observations were always made for tumors, both gross and microscopic. Tumors developed in this study, but the incidence and kind of tumor did not vary from the control animals. When we reviewed the incidence and type of tumors formed in our control animals for the past fifteen years (a considerable number of thousands), there was apparently no difference between the incidence and types of tumors reported in this particular group of three studies, and those we had previously found in our colony. I would say that there is probably no carcinogenic hazard on ingestion from this compound.

DR. HUEPER: Shubik reported dodecylbenzene sodium sulfonate to be a cocarcinogen.

DR. NELSON: The Shubik experiments were made with surface application of this compound and not injection.

CONFERENCE ON "PHYSIOLOGICAL ASPECTS OF WATER QUALITY"

REVIEW SESSION

MINERALS AND TRACE ELEMENTS - SUMMARY STATEMENT

DR. McKEE: The purposes of this meeting have been many fold, and I should like to point out that they include intentional cross-fertilization between physiologists, medical doctors, biologists, and even some entomologists, engineers, and chemists. It is to be hoped that this introduction to the subject, and the opportunity to bring you together will lead to correspondence between participants.

In this afternoon's session we are to have three summary statements presented. The first summary statement is to be given by Dr. James L. Whittenberger, Professor of Physiology and Public Health, School of Public Health, Harvard University, who served as Chairman of Session I.

Statement on Minerals and Trace Elements

DR. WHITTENBERGER: The subject of the first session of this conference was the occurrence and physiologic effects of minerals and trace elements.

Our keynote speaker, Dr. Kehoe, set the stage for the session in his remarks about the composition of biological fluids, and on the complex equilibrium of body fluid components existing between the internal environment of the body and the external environment as represented by food, water, and air. He pointed out the evolutionary origin of body fluids from sea water, the composition of "physiological" fluids being regarded, with considerable accuracy, as dilute sea water.

However, when one goes beyond these generalities to look for answers to the questions being asked at this conference, one finds relatively few specific answers and many areas of ignorance. What determines the relationship existing between substances in the water and food and their presence in the body?

We know little about the quantitative aspects of absorption of materials from the gastrointestinal tract. Although a large area is available for diffusion and active transfer, the intestinal mucosa

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is, by no means, a simple membrane. Mucus secreting glands form a protective coating over much of the surface. The pH varies over a wide range and numerous enzymes are present, derived both from gastrointestinal secretory glands and from the microorganisms normally present in much of the intestinal tract. The transfer of material across the surface epithelium may involve transport mechanisms within the cell which are often highly specific.

Incidentally, I think some of the differences in toxicity between mammals and fish may be due to the fact that the fish has a very different contact with the water environment. He satisfies his respiratory needs through the dissolved oxygen and dissolved gases in the water. But, at the same time, the fish is exposing a large membrane surface to all other elements that are present in solution in water.

After absorption, materials travel through the portal system to the liver, which is well known for its capacity to effect chemical transformations of many kinds. Noxious chemicals are likely to be inactivated here by hydrolysis, conjugation, or other mechanisms.

A secondary line of defense is the kidney, which filters plasma at the rate of 7.5 liters per hour, has a remarkable ability to eliminate "unwanted" chemicals, and to reabsorb the needed ones, such as water and sodium. Only about 1 per cent of the water filtered in the glomeruli is eliminated from the body.

The session was devoted mainly to what are called trace elements, characterized usually by low concentrations in the body; and, perhaps more importantly, by difficulties of identification, both in terms of chemistry and in terms of their functional activity in biological systems. Lack of knowledge of the possible role of trace elements in health and disease has left the possibility of speculation.

Over the past half-century, many theories have been proposed to explain senescence, atherosclerosis, cancer and other diseases on the basis of the presence of trace elements in body tissues, or even in the rivers, soil and vegetation of the area of residence. A few of these associations have been found to be causally related, expecially for lower animals and plants. A number of examples were referred to by the speakers.

As in any field of research, advances depend upon improvement in methods and instruments of investigation. In addition to epidemiologic methods, balance studies and dietary manipulations, studies of tissue enzyme activities have recently proved of much value in this area of research. Improvements in spectrographic methods have been especially important in study of the function of trace elements in enzyme activity, the mechanism of much of the biological activity of these elements.

Several of the papers presented illustrated methods for demonstrating a normal biological role for a trace element, the prevention of metabolic defect, changes in growth rate, prevention of anemia, and effects on enzyme kinetics.

Speakers also emphasized the importance and the difficulty of obtaining truly reliable information about trace elements in tissues and their function. I will not attempt to summarize the specific presentations since these are already available. I shall mention only some of the main principles as I understand them. Some principles applying to trace elements are the same as those applying to nutrients, drugs, and other substances. That is, the organism can suffer from a deficiency as well as an excess of an element if the element has a biological role. Even if it does have a biological role, an excess may be toxic.

Dr. Monty's paper illustrated some types of toxicity, as well as some problems of studying interrelationships among dietary components. Excess molybdate interferes with the growth rate of the whole animal and lowers the sulfide oxidase concentration of liver, possibly by its effect on food intake. Decrease of the enzyme may explain the induced copper deficiency, since copper is presumably rendered insoluble in tissue by combination with sulfide. The copper deficiency, in turn, leads to anemia because of the role of copper in blood formation. Addition of a sulphur-containing amino acid to a molybdate-poisoned animal may restore the growth rate, but may aggravate the anemia if the animal is on a low copper diet. I think this at least illustrates the complexity of some of these interrelationships.

Dr. Stokinger described several activities of vanadium in biological systems, emphasizing the ability of vanadium to inhibit the synthesis of cholesterol. He pointed out the experimental association of cholesterol inhibition with a decrease in the amount of atherosclerosis in the vessels of the experimental animal and called attention to further epidemiologic investigations in man along the lines suggested by these laboratory studies.

Dr. Anderson, in subsequent discussion, emphasized the need for extreme caution in interpreting associations between diseases and specific environmental variables, such as the recent association described between the hardness of water and certain types of cardiovascular disease. It should be recognized that innumerable other factors exist which, if looked for, may account for peculiarities in geographic distribution.

In summary, you have heard examples of numerous kinds of investigation of the effects of trace elements in the body. Each of these elements has its own particular requirements and complexities and difficulties and, at the same time, obtain information which will be helpful in solving our problems. I know the biologists have profited by being here, getting new points of view, and learning about some of the problems in environmental health.

INSECTICIDES - SUMMARY STATEMENT

DR. MCKEE: The second session of this conference, on insecticides and organics was divided into two parts. The chairman of the session on insecticides was Dr. Arden R. Gaufin. Dr. Gaufin is with the Department of Zoology and Entomology of the University of Utah.

Statement on Insecticides

DR. GAUFIN: At the present time insecticides are among the most important of our water pollutants. They occupy somewhat a dual role. Pesticides promote health directly through the control of vector-borne diseases, and indirectly through increased and improved agricultural production.

On the other hand, the careless use of insecticides, or the excessive use of such materials, may destroy very valuable resources; both aquatic resources as well as some terrestrial resources. In the United States alone, the annual production of synthetic organic insecticides, which were essentially unknown before World War II, reached the level of some 569 million pounds in 1956. Although something less than 200 basic chemicals are really important as pesticides, the situation is complicated by the fact that there are thousands of different combinations employed.

Dr. Hayes pointed out that there are possibly some 90,000 different insecticide forumlations. Not only do we have to deal with many different formulations, but the extent of usage of these insecticides, is vast. Some 30 million acres of crop land are being sprayed every year, as well as some 6 million acres of forest that are being treated by airplanes.

The harmful effect of these poisons on human populations to date evidently is rather slight. The death rate from poisoning caused by all pesticides in the United States is slightly less than

.09 per 100,000 population. Dr. Hayes pointed out that, strangely enough, most of these fatal accidents are caused by the older pesticides; not by the relatively new ones.

Now, if we consider morbidity rates in contrast to mortality rates, there is one study which indicated that the ratio of fatal to nonfatal cases associated with poisons was 1 to 13. Evidently, then, the effect on human populations is not too serious.

Dr. Hayes, furthermore, pointed out that nothing is now known to justify the suggestion from some sources that pesticides currently constitute a serious threat to human health. However, individual cases of such conditions as purpura, anemia, etc., may be caused by specific insecticides. The amount of these insecticides present in human beings is far greater than we realize. I think Dr. Hayes stated that the concentration of DDT in every individual ranges between 1 to 10 parts per million, but that the concentration is limited by an equilibrium which eventually results in the excretion of excess amounts of these insecticides.

If we consider the specific action of these insecticides, particularly the organic phosphates, on human beings, we find a rather definite pattern. Dr. Fitzhugh pointed out that these organic insecticides act to inhibit cholinesterase. The first symptoms are weakness, blurred vision and nausea, followed by vomiting, cramps, difficulties in breathing, eventually coma and convulsions. Death, in most cases is due to a respiratory failure.

Generally, the chronic effects of exposure to insecticides are not known too well. Rather, the effects are more or less immediate. Dr. Fitzhugh also pointed out that if two organic insecticides are used together, the effects are much more severe than from one alone, particularly if there is an interval of a few hours between exposures. For example, he stated that when insecticides such as dellnave and malathion are used together, and the individual is exposed to malathion four hours later, the death rate is considerably increased.

In the final paper dealing with insecticides, Dr. Hoffmann pointed out that with the widespread use of insecticides, many of these are likely to get into water resources and cause rather severe kills of aquatic organisms. However, he pointed out that in the United States alone, there are some 10,000 species of

.09 per 100,000 population. Dr. Hayes pointed out that, strangely enough, most of these fatal accidents are caused by the older pesticides; not by the relatively new ones.

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In the final paper dealing with insecticides, Dr. Hoffmann pointed out that with the widespread use of insecticides, many of these are likely to get into water resources and cause rather severe kills of aquatic organisms. However, he pointed out that in the United States alone, there are some 10,000 species of injurious insects. Those injurious insects may cause crop losses of approximately $4 billion annually. Certainly, the control of these insects justifies the use of many insecticides -- in fact, make their use absolutely necessary.

As I pointed out earlier, however, there are very distinct differences of opinion. Entomologists, and agriculturalists are interested in controlling injurious forms. Fish and wildlife people are interested in conserving beneficial water resources. The problem is to arrive at a point where we can control the injurious forms, and yet at the same time cause minimal losses to beneficial forms.

In order to meet this need, I am sure that a great deal more cooperation will be necessary between various agencies. The fish and wildlife people must be aware of the objectives of the entomologists, and the entomologists must necessarily take into account that severe fish kills can occur if insecticides are not properly applied. There have been a number of cases of rather severe losses of aquatic life due to the use of insecticides. For example, in the Yellowstone River during 1955 about 95 per cent of the aquatic insects were wiped out by use of DDT. Some three months later, there was a fish kill. It has been considered that this fish kill was caused by starvation of the fish due to the earlier loss of insects. Dr. Hoffmann has discounted that possibility.

One thing that makes the loss of these aquatic forms less serious than might appear on the surface is the fact that most aquatic invertebrates have an amazing reproductive potential. The population can return rather rapidly. In fact, in Dr. Hoffmann's studies, it was found that while as many as 95 per cent of the organisms were lost, the population had practically reached its former level in a matter of three or four months.

It was also pointed out that the effects of these insecticides upon fish are quite different than upon other animals; in fact, they may be much more serious. Many fish actually store rather vast amounts of these insecticides. What the long-term effect of this may be, no one knows. Certainly, this is a field which will require much research before we know all the answers.

In order to ascertain their effects when these materials are used in the field, we need to know something about the ecological differences between ponds, lakes, and streams. We have to know something about the species of fish involved and their reaction to specific poisons. We should also know something of the life history of the organisms concerned.

For example, if an insecticide is applied to an area in late August, when most of the aquatic insects have emerged, the results may be quite different than if the insecticide were applied two months earlier. Another definitely important problem is the fact that many of these insecticides may be stored in bottom muds or in algae on the bottom, since this material might be released later and have a deleterious effect.

This is a field, then, in which we know very few of the answers. We know what is occurring. We realize that the use of these insecticides is important and, on the other hand, that these insecticides can cause severe distrubances in nature. In order to solve the problem, I am sure that we must have a cooperative approach between various agencies, and we will need much more research in order to save some valuable aquatic resources.

ORGANICS - SUMMARY STATEMENT

DR. MCKEE: The chairman of the session on organic substances was Dr. Norton Nelson, who is in the field of industrial medicine and occupational health at New York University Medical School.

Statement on Organic Substances

DR. NELSON: I shall not attempt to present a detailed summary of the several papers. In order to do them justice, one must go back to the printed material. I would prefer to give a somewhat personal reaction to these papers.

One of my initial reactions to the session, and I think one that perhaps will remain as a predominant impression, is the timeliness of this conference; especially the timeliness of our examination of the problem of organics. Although the trace element problem has been with us a long time, it may be overshadowed by the impact of chemical synthetics in a wide diversity of organic compounds, some of which are being discharged to our water resources.

I think that the three quite different papers presented in this session pointed to the state we are in with respect to public health knowledge of the importance of this class of compounds; and, further, if they are significant, how to deal with them. Obviously, such a review as we have had here is only a beginning and a red flag. It is perhaps most useful to the extent that it does warn us to begin to do research now, while we have a chance, before the problem reaches dangerous dimensions.

Dr. Zapp in his discussion called attention to some very important issues, pointing out that what really is required here is a rational understanding of the physiological effect of these

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materials; since virtually without exception it has been possible in the past to establish an essentially no-effect level. That is to say, there is a finite contamination level, which can be defined if we know enough about it, and which will produce no significant health impairment over the life span of the individual.

I think this is a point that must constantly be kept in mind, and that all our work should be aimed toward being in a better position to define the no-effect level. Without it, we are at the mercy of those who are properly concerned, but who might blindly stop all industry and all technology.

Dr. Zapp also pointed out, and I think it became apparent in Dr. Hueper's talk as well, that the current exception in the food and drug laws which apply to carcinogens has no basis in present experimental fact. When knowledge is sufficient to permit the definition of safe levels of materials which are carcinogenic under some circumstances, than perhaps we can take a more rational approach to this problem as well. Until such time, obviously, great caution is in order.

Dr. Paynter's contribution is important from several standpoints. One point is that it informed us -- reassured us, if you will -- as to the lack of evidence that dodecylbenzene sodium sulfonate will produce significant physiological damage, and the data presented are encouraging in this regard.

It seemed to me, in addition, that his was a useful presentation to bring before this group, which represents so many different disciplines. His paper is an illustration, or a prototype, of the type of detailed, lengthy and elaborate physiological studies that are required in the investigation of water contaminants. This study has occupied a large staff in a number of different laboratories over a long period of time, and a great deal of money has been expended. I am sure it does not even yet provide all the answers.

If one looks ahead to the possible necessity of subjecting all organic water contaminants to the same degree of refined testing, it is not very difficult to see that this pursuit could conceivably engage a very large proportion of our scientific talent. It might well be that what we need are more efficient ways of dealing with the evaluation of chronic toxicity. New techniques may be needed in order that our research staffs will be able to devote themselves to other urgent health problems, rather than to go through this elaborate and (without any reflection on Dr. Paynter's work) cumbersome procedure.

Dr. Hueper, in discussing cancer, touched on a problem that is of great current concern. It has recently been reviewed from the standpoint of legislative implications, in the Miller Amendment and again in the food addatives law. I think all of us feel that this is an area in which we are not as reassured as we would like to be. It was somewhat encouraging that Dr. Hueper was able to tell us there is little actual evidence that our water supplies are a significant source of cancer in the human population.

On the other hand, he equally pointed out that there are possible threats which have not been looked at in such detail to resolve the questions that legitimately arise. He emphasized that much more information must be acquired before we feel confident that this source is not or cannot become significant.

One of the reasons for calling this conference was to examine research needs and consider what should be done in the future. The needs include not only specific research projects, but questions of surveys, or reorientation, and legislative programs. I can make no contribution to the last of these points, but it does seem to me that certain needs can very definitely be recognized here; and, further, that those who are competent to deal with and attack these research problems be emphatically encouraged to do so.

In this sense, those who have a primary interest in analysis and surveys - as does Mr. Middleton - are an essential reinforcement to those who have purely physiological interests; the two areas can't really be separated. It is clearly impossible to examine all possible contaminants for physiological effects, and it is clearly impossible to analyze for all conceivable organic substances in water; there must be a close interplay and feedback between these two interests.

It seems to me Mr. Middleton's paper pointed out that a survey program, based on analytical determinations directed toward specific safety questions and dealing with specific organic contaminants, has much greater value rather than one directed to the determination of organics in general or to classes of compounds. It is rather generally true, and I think this has become evident in the session on organics, that you cannot judge the physiological effect solely on the basis of the class of compounds to which a chemical belongs. This applies whether one is concerned with carcinogens or other kinds of toxicity. Accordingly, there must be a close orientation and integration between the survey program and the physiological studies.

One can attempt, through the epidemiological method, to answer the question as to whether present contaminants in water do or do not produce health defects. I believe that it is true that little application of the epidemiological method has been directed toward water resources, insofar as physical or chemical contaminants are concerned. Actually, the water sources of contaminants will often be more easily investigated than will other sources of contaminants. By this, I mean merely that the water intake is only one source of entry into organisms of those materials we are concerned with here; the food supply and the air contribute as well. Food and air sources of contaminants are more difficult to measure and to quantify than water sources. Nevertheless, the focus on water is an important one, and one that should be pursued.

Lastly, it seems to me that there is an urgent need for more efficient and informative ways of answering problems concerning the health hazards of contaminants. It is perfectly true that the pattern that has developed through the Food and Drug Administration testing program has worked fairly well. Nevertheless, it is at its best cumbersome, expensive and time consuming. At its worst, in my opinion, it fails to give the kind of reassurance that I think we must insist on -- where lifetime ingestion of trace contaminants is in question.

For these reasons, and this statement applies not only to water pollutants but to the whole field of chemical toxicity, I think we need a searching reexamination of our ways of evaluating toxicity problems and better means for predicting safety. I think such improved procedures may well arise out of a close integration between the approach described in this conference, in which one is dealing with basic mechanism of action, and that in which the approach is the applied one of establishing standards for safety of contaminants, whether they be in food, air, or water.

NATIONAL AND INTERNATIONAL STANDARDS OF WATER ANALYSIS

By

F. W. Gilcreas

Over the past years criteria for safe and satisfactory supplies of drinking water have been discussed, considered, established, changed and improved. Such standards, to be effective, must be accompanied by accepted methods of analysis and these methods must change and develop with changing concepts of standards of quality.

The need for recognized methods of water analysis was considered many years ago - as early as 1888 in the United States - and discussions of the problems involved lead to the publication of the first edition of Standard Methods of Water Analysis in 1905. During the ensuing years successive editions of this manual have appeared, keeping in step with developments in water treatment and basic ideas of water quality. Late 1960 will mark the appearance of the 11th Edition of Standard Methods as produced and published by the APHA, AWWA and WPEA.

Changing Concepts of Water Quality

The changes in our ideas of water quality are in many ways illustrated by the content and extent of these previous editions. The 141 pages of the first edition have expanded to about 650 pages in the 11th to accommodate the new technics required by present treatment of water and waste water. Comparable changes have appeared in the content of the book - as for example, the 2nd Edition included methods for the detection of pathogenic bacteria in water - a section eliminated from all succeeding editions as emphasis on this subject gave way to emphasis on good and safe sanitary quality.

The 11th Edition will contain a specialized section on the measurement of radioactive pollution in water - required by the rapid advances in nuclear science. The important influence of biological parameters of water quality is recognized in greatly expanded sections relating to biological and bacteriological examinations and by a separate and expanded discussion devoted to iron and sulfur bacteria.

New Methods of Chemical Analysis

The section relating to chemical analysis of water, a section which applies directly the theme of this conference, contains many new and revised procedures to conform to the rapidly developing concepts of water quality and in particular to the need for more sensitive methods for the accurate determination of trace amounts of many elements that may be found in drinking water.

It is interesting to note that at the time of the first edition, apprehensions were expressed that standardization of technics and methods would stifle progress and research both in analytical methodology and in water quality and treatment. Fortunately these fears have not materialized.

The use of the designation "standard" to describe these accepted methods is often frowned upon as implying action by a superior authority and the use of some less definitive term - such as approved or accredited - has been recommended. Primarily for the purpose of adhering to tradition, methods for the examination of water will continue to be known as "Standard Methods." The international methods, developed under the auspices of the World Health Organization are however, designated as "approved methods," primarily to avoid confusing methods with criteria of quality.

Standard and Tentative Methods

Two types of methods should be recognized, those which through detailed study and long usage have been found to be fully reliable, accurate and precise, and those more recently developed and requiring further study to establish their reliability and yet of demonstrated application to specific analyses. The first are given the status of "standard" and the second that of "tentative." A conservative point of view in the acceptance of a given method and its designation as "standard" or "tentative" is essential if the published volume of methods is to be dependable and useful. However, the development of methods for the examination of water must parallel the development of procedures for water treatment and must likewise parallel changing concepts of water quality.

It has often been said that production of analytical methods should follow developments in quality and treatment. But this could not be - rather they should progress in parallel; it is impracticable to establish standards of quality unless procedures for measuring quality are available.

A major criticism of the idea of published Standard Methods has been that - once printed - they are unchangeable and improvements in specific technics and new and tested methods cannot be added until a new edition appears; an average time of five years; although twice nearly ten years elapsed between successive editions.

To overcome this pertinent objection the Joint Editorial Board for Standard Methods has developed a procedure whereby - by publication in one or all of the national journals of the associations sponsoring Standard Methods - a tentative method can be changed to standard, and new and authenticated technics added. No method may be deleted or degraded during the interval between editions.

International Standard Methods

Although the principle of establishing standards as accredited technics was perhaps first accomplished in the U. S., other nations have undertaken similar programs to conform to the requirements of water treatment and quality in their own areas.

Thus in Japan a volume of Standard Methods for the Examination of Water has been published and similarly a companion volume has been developed in Korea, both to meet specific needs of each of these areas. Other countries have done the same in whole or in part. The United Kingdom has been active in this field for many years and the several editions of Bulletin 71, "The Bacteriological Examination of Water Supplies" published by the Ministry of Health and the Ministry of Housing and Local Government, are well known and widely used throughout the world.

Four years ago, the World Health Organization undertook to consider the development of international standards of quality and international methods for the examination of water which could be used as a basis for controlling the quality of water, particularly that used in international travel. Some two years ago a volume of standards of quality and what are designated accepted methods of analysis was published. There have been many criticisms of that report. It certainly is widely different from the United States concept of what constitutes standards of quality. And in many instances, the method for examination vary to a marked degree.

Analytical Methods Conform to Needs

The answer to these criticisms, of course, is this: The analytical procedures were established to conform with the standards of quality which were established. In many cases, although the criteria of quality may differ widely from those of the United States which we understand and have used for so many years, nevertheless, they conform to the needs of the world-wide problems of the time. And that is what the volume of Standard Methods in this country must continue to do for many years to come.

The establishment of Standard Methods and the general acceptance of these has been a potent force in the improvement in water quality and treatment over the past 55 or more years.

At this conference many new ideas regarding water quality and the physiological factors relative to many chemical compounds found or appearing in water have been discussed. These discussions will have a major influence on our ideas of what constitutes "good" water and will effect proportionately research into methods of water analysis and the development of new technics. In many instances the procedures in the 11th Edition will be adequate at least for a few years. In many they will not be, and then significant research will be necessitated.

Future Needs in Standard Methods

In the 11th Edition there are many new and important changes and improvements. Developments in the significance of many aspects of water quality will undoubtedly render the 12th Edition of the APHA Standard Methods as different from the 11th, as the 11th is different from the first. Future editions will need to include swift and rapid tests for the control of water quality, as has been fundamental in all past editions, and the more complex technics requiring expensive instruments and skilled operation to service the research and detailed analyses that will be essential for the expanding field of water quality.

International methods of water analysis will develop at the same or perhaps an accelerated rate and in the same or comparable directions.

It is hoped the next 5 years will mark a major advance in the methodology of water analysis to define and to measure water quality.

REPORT ON RESEARCH PROJECT AT UNIVERSITY OF PITTSBURGH

A contributed report summarizing research under way at the Graduate School of Public Health, University of Pittsburgh

By
Prof. M. A. Shapiro, Dr. W. H. Hill
Dr. F. A. Rosenberg, Dr. G. Fred Lee

To our knowledge, no major studies other than the one currently in progress have concerned themselves with the physiological effects of trace elements present in the finished drinking water of a community. As distinct from the daily intake of food, where maximum allowable concentrations of certain trace elements have been fixed, drinking water can contain these substances in quantities which may prove harmful to the human organism, not in an acute sense, but through concentrating in certain organs of the body with subsequent pathological changes.

The city of Pittsburgh obtains its drinking water from two separate sources, namely, the Allegheny and Monongahela Rivers, which enables us to compare the relative concentrations of elements present in the water supplying two distinct populations, as well as relating these findings to the work of other authors, particularly where various municipalities have been studied.

The project as originally conceived visualized an approach to the subject from three angles: analyses of drinking waters used in the Pittsburgh district in order to ascertain what differences exist with respect to their trace metal and organic materials content; analyses of biological materials from autopsies and biopsies performed in the University of Pittsburgh Medical Center; and an epidemiological study of the mortality of the Pittsburgh population. A possible fourth angle was also considered: analyses of foodstuffs normally consumed by the population with respect to those elements which water and tissue analyses would show to be of particular interest in this study. It was felt that in this manner it would be possible to discover what relationship, if any, exists between the drinking water intake and the well-being of the Pittsburgh population.

Analyses of drinking water

Grab samples of treated Allegheny and Monongahela water were analyzed by emission spectroscopy after evaporation to dryness. There were very considerable differences of the two waters with respect to a number of elements, such as aluminum, barium, copper, iron, strontium and titanium, and lesser, but still significant, differences with respect to cobalt, and vanadium.

On the basis of these results it was decided to analyze water from three area water works (two treating water from the Allegheny River and one from the Monongahela River) regularly, at weekly intervals. The samples are collected at the water works in the course of daily routine sampling by the plant staff, and represent composites of all samples taken during a week. The recovery of the solids dissolved in these water samples was attempted in a number of ways, with evaporation in glazed silica dishes finally being adopted for reasons of minimum contamination and ease of removal of the evaporation residues.

Samples have been collected for the past 32 weeks (as of late 1960). It is intended to continue the program for a total of about two years in order to ensure that the hydrologic parameters such as high and low flows in the rivers, spring floods, abnormally dry or wet seasons, and effect of low flow augmentation are properly reflected and evaluated. For reasons of economy and efficiency, the dry evaporation residues of these water samples have been subjected to emission spectroscopy in batches of 20-30. These analyses have shown that consistent differences are already discernible. Details will be published when a larger body of analytical material has become available.

The decision to obtain these analyses on the waters leaving the plants rather than on waters at particular points on the distribution system was based on the desire to avoid uncertainties introduced by unknown transmission lines which could give rise to trace metal contamination. That this decision was a wise one is borne out by the fact that water samples taken occasionally from the tap in the laboratories where this work is being carried out have shown a very high copper content, manifold that of the original water from the plant for the same periods. This observation raises the question whether the present work pattern of analyzing water works water samples should not be augmented by analyzing water from various points of consumption, choosing e.g., new housing districts with presumably modern water mains and house lines, and old ones with probably quite different line materials. It is one thing to produce the purest water possible and something else again to have it contaminated in transit to an extent that water works analyses might become far less meaningful. This problem will be given very serious consideration.

Spectroscopic Methods of Analysis

One comment on the present method of spectroscopic analysis would seem in order. The results are semi-quantitative and embody a relatively large margin of error. This is inherent in the normal method of analyzing for trace elements in the presence of overwhelmingly large amounts of the major elements. With the ratio of these major elements now known, future analyses will be made on the basis of using the major elements as matrix materials so that much more dependable figures for the trace elements should be obtainable. Also, it is contemplated to "concentrate" trace elements by means of precipitation with a "universal reagent" composed of Thionalid, (thioglycolate of aminonaphthalid), tannic acid, and 8-hydroxyquinoline. These refined analyses will also be made on the older water samples because there is enough material left for the purpose.

In an effort to learn something about the composition of Pittsburgh waters in years gone by, we have extracted sands from the slow sand filter beds of the Pittsburgh water works. Some of these beds have been in operation for some 30 years. The extracts will be analyzed in the same fashion as the regular water samples, but the results will be evaluated only as far as ratios of the various elements are concerned since the quantitites of water, that have passed through those beds, is not known. However, a comparison of the ratios with those obtained on present water samples may be quite illuminating.

Concentration by Ion Exchange Resins

A second, and different, approach to the problem of water analysis was made on the basis of ion exchange resins. It was thought that for the purpose of accumulating large average samples, and avoiding at the same time the evaporation of large quantities of water, it would be preferable to pass the water to be sampled and analyzed through mixed bed ion exchange columns. With properly adjusted flows, continuous and dependable samples could be taken over any reasonable time interval, and the total sample could be just as small or as large as would be desired. While the normal strong acid cation exchangers and strong base anion exchangers will pick out of the water any and all cations and anions, there are special resins available which have specific affinities for certain ions, and further modifications are possible by appropriate manipulation of the resins, e.g., degree of saturation with ions.

Considerable work has been done in these laboratories to date in an effort to delineate the best conditions for the practical use of such resin systems, and the water used for the purpose has been that delivered at the laboratory tap. A relatively small column, with a resin packing of a pound or two, will demineralize some 200-300 gallons of water, depending on the solids content of the water. Taking the sample in this manner does not seem to present a problem, through it is not established yet at what degree of saturation of the resin the sampling should be stopped. A conductivity bridge is customarily used to follow the progress of sampling.

The real problems of the method revolve around the elution of the ions from the column. This is a tedious process, and the quantity of eluate can be very considerable. Elution may be performed on the mixed resin, but this has brought on such difficulties as precipitation in the columns. The mixed resin can also be separated hydraulically into the cation and anion fractions, with high efficiency if a 20 per cent glucose solution is used for the purpose. The various fractions of eluates obtained so far from mixed and separated resins will be analyzed (after evaporation) to see at what point in the elution process we could advantageously stop and still obtain recovery of substantially all of the elements of interest.

Recovery of Elements from Resins

In future work we will also consider the possibility of eluting from charged resins particular elements, or groups thereof, by either special buffer systems, or sequestering and complexing agents. This could be a very fruitful approach and it is known to be operative in related fields of endeavor. We ourselves have made the observation that certain metals, presumably copper, nickel and cobalt, come off the columns in definite fractions when eluting with dilute ammonia water.

Instead of eluting the ions from the charged resins with acid and alkali respectively, we have also contemplated dissolving them by destroying the cross-linking, which makes them insoluble, by means of strong caustic and hydrogen peroxide. Orienting experimental work has shown this to be entirely feasible, and the method may thus provide the tool for taking aliquots of a charged resin, while the sampling of the solid resin would entail the customary difficulties attached to any and all sampling of solids.

There are some other important aspects of the analyses of water with ion exchange resins. It allows interpretations of the kind of compounds contained in the water beyond the mere analysis for an element. For instance, emission spectroscopic examination of the evaporation residue of water tells us what elements there are, but it will not tell us whether chromium is there as the hexavalent chromate or the trivalent chromic chloride (or sulfate). The resin columns would make such differentiation for a number of elements that may be of importance. Resin treatment will also tell us something about at least some of the organic constituents of water, such as ionic surfactants. Sodium alkyl benzene sulfonate e.g., would be retained on the resin columns and could probably be isolated therefrom and brought to analysis without much difficulty. This is, of course, not the case for the spectroscopic analysis of a water evaporation residue.

We would expect to find phenolic and chlorinated phenolic materials on the ion exchange resins, and presumably in an unaltered condition. This would unlikely be the case for the materials extracted from water with activated carbon. In like manner, basic organic materials, such as pyridine, would be found on the resin columns. Even ester-like organic compounds, such as some of the surfactants, might be split and fixed on resin columns because the individual resins are strong esterification and saponification catalysts. Resin treatment of water could therefore be a very versatile tool for the study of inorganic as well as organic constituents.

Separation of Inorganic and Organic Constituents

In our studies on the physiological aspects of drinking water quality, we have in the past paid particular attention to the inorganic constituents, but not to the exclusion of the organic ones. We have contemplated a combination of ion exchange resin treatment, which would pick up the ionic materials, and activated carbon, which then would remove the non-ionic organic compounds. This approach could be particularly fruitful because the grand mixture of organic materials on the activated carbon in the work carried out by other investigators could undergo resinification with resultant difficulties of recovery and identification, while a combination of ion exchange resin and activated carbon treatments would keep the organic materials separated from the beginning in three general groups: acid, basic and neutral. In addition, this combination treatment should lead to more complete recovery of the organic materials than would seem possible with activated carbon alone.

We have carried out additional work in connection with organic constituents in water. During our ion exchange work we observed that the saturated resin columns seemed to contain appreciable amounts of organic materials in the form of oily droplets. It was not clear whether the retention of these organic materials was due to a mechanical filtering out of oil from the water stream by the rather densely packed resin charge, or whether special physical affinities of the resins for certain organic compounds was involved. In an effort to clarify the picture, a saturated resin charge was divided into a number of aliquots, which were then allowed to dry on watch glasses in the laboratory atmosphere, and were thereafter extracted in Soxhlets with several solvents (one for each aliquot). The work is not completed yet and no identification of the organic materials extracted from the resin aliquots has been made so far.

Analyses of Biological Materials

The subject of analyses of biological materials has not advanced beyond the thinking stage as yet. This delay has been a planned one because it did not seem advisable to undertake such analyses without prior knowledge of significant differences among Pittsburgh waters. To be sure, normal levels of elements in various well and diseased tissues would be obtained, which then could be compared with such levels from other parts of the country as are known from literature, and this will be done. However, the danger of accumulating a mass of unusable data is great, for no useful purpose would seem to be served by obtaining such normal levels for large numbers of elements, because only a very few of them will bear a significant relationship to Pittsburgh waters. It is for this reason that we delayed actual work on biological materials and rather concentrated on the development of proper methodology.

Serious thought was given particularly to the problem of "ashing" tissue, preparatory to analysis for the various elements. Dry-ashing in a crucible involves hazards of losses and contamination, and moreover is burdened by incomplete combustion. If platinum crucibles or dishes are used, the investment is heavy. Wet-ashing with strong acids involves also possible losses, it is tedious, and it requires a lot of hood space, not to speak of the destruction of the hoods and the duct work. Alkaline digestion, as developed by one of our group, is feasible in many cases, avoids losses, can be carried out in the open laboratory, and is relatively fast, but it is burdened, like acid digestion, by contamination from the chemicals and the vessels used.

The only sure way of avoiding losses and contamination, and to do the ashing job with a minimum expenditure of time, is the combustion of the tissue, dry or wet, in a Parr Oxygen Bomb or possibly by the Schoniger Oxygen Flask method. The former has been investigated and developed by one of our group for another purpose, but the latter is untried as far as we are concerned. The Parr Bomb method will be applicable in all cases where the available methods of analysis are sensitive enough to determine a given element with sufficient accuracy at the level at which it is found in human tissue and with the amount of sample burned in the bomb. Several burnings in the same Bomb may be necessary to reach the desired level. Where the content of a tissue with respect to the given element is too low to permit application of the Parr Oxygen Bomb technique from a technical standpoint, one of the other ashing methods would have to be resorted to. It would seem then that a prudent choice of the proper ashing method for each element would permit a more rapid accumulation of dependable data than would be possible if just one method were decided upon as being applicable to all cases.

Analysis of Trace Elements in Tissues

The pattern of work to be undertaken may thus develop as follows. Orienting work concerning ashing method and analytical procedure will be undertaken with tissue from the hospitals of the University of Pittsburgh Medical Center, without special regard to whether the people were well when they died or not, as long as medical and general background information is known about the people to permit later evaluation of a variety of factors. Initially emission spectroscopy will be used as a scanning tool to tell us what elements might seem of particular interest either because their levels are much higher or lower than those encountered in other cities and available from the literature, or because there seems a striking difference between well and diseased tissue or between people from various areas of the city. Eventually we will have to decide whether elements that show up in a meaningful ratio in the separate water supplies should be the ones with which we should concern ourselves most, or whether this list should be augmented in any way by elements which showed up notably in the general survey analyses.

Numerous references may be found in the literature relating to the essential nature of certain trace metals in metabolic processes. Whereas the majority of these studies have dealt with the indispensability of these elements in their relation to various enzymes, and thus with the effects of a deficiency of these cations as regards the steady state, a number of authors have attempted to determine the relationship of an excess of certain metals to pathological conditions.

These studies have generally been attempted in two ways. The first has been an effort to establish base lines for the presence of trace elements in normal tissue and then to compare these findings with the results found in the tissues of individuals suffering from various pathological conditions. The second has concerned itself with a study of a specific cation and its effects when present in the tissues in varying quantities. Considerable time and effort have been spent in reviewing the literature and determining the nature of the studies of various authors. Whereas a series of papers by one investigator includes an analysis of up to thirty trace elements in twenty-three different tissues, the majority of other authors have limited themselves to fewer elements, particularly when comparing their presence in diseased and normal tissue.

Analyses of Foodstuffs

We would be seriously at fault if we were to neglect in this project the food intake of humans. After all, many plants concentrate certain elements preferentially, as do animals, and modern processing methods of foods cause the introduction of certain metals and metalloids into the prepared foods, and they cause the elimination of other elements. We may well find the intake of certain elements through foods to be very much higher than anything that could conceivably be derived from the water consumed by a person. Otherwise noteworthy differences might thus be completely obliterated.

It would seem necessary then to obtain reliable analytical data on the composition of "standard" foodstuffs with respect to their inorganic constituents. Such data is not now available, or only to an insufficient extent, because the biochemists and nutritionists of the past decades were interested primarily in the major elements, the trace elements assuming importance only in the very recent past, and then one by one, as they became recognized to be vital for human nutrition. For this reason the literature on this subject is spotty and will have to be augmented by work of our own. The first requirement of such a study might well have to be an investigation of the dietary preferences of local population groups before we could say with reasonable confidence what constitutes "standard" foodstuffs.

CONFERENCE EVALUATION

By

Dr. Abel Wolman

Attendance at any symposium has always been a traumatic experience in that it is a sobering situation for several days to sit and listen to a rehearsal of my own ignorances, upon which are superimposed the ignorances of a very large number of participants in the symposium. Similar experience has led the Planning Committee of this Conference to the suggestion that perhaps it would be valuable to examine the symposium and what it might mean to an observer.

What kind of an evaluation does the committee hope to have? It would be necessary to decide: An evaluation by whom and for what purposes? Was it to be an evaluation by the man on the street who stumbled into this kind of a conference and who ordinarily gets most of his scientific information from Drew Pearson's column? Or, coming a little closer to home, was the evaluation of the symposium for the benefit, say, of a Congressman? Or is it to be an evaluation on the part of a practitioner, as I happen to be, in the field of sanitary engineering, and the environment.

A combination of all these points of view may lead to an assessment of the subject matter in the last two days, dealing with methodology, with facts, with concentrations, and with what the future may hold.

Water As a "Natural" Environment

Certain conclusions are quite obvious, before one reviews the positive and negative values of a conference such as this. First, the natural environment of water, to which we have added insults over the centuries, has - in fact - never been a natural environment. This situation has been pointed out, of course, by almost every speaker. We fall into a nomenclature that indicates we are adding insults or stresses to some theoretical natural environment.

One interested in the natural environment throughout the world knows that there are no parallelisms between any two sections of the world, geographically, geologically, industrially or otherwise. So these insults or stresses are not applied to a common base.

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A corollary of this observation is that we will never have, by definition, a natural environment anywhere. We had assured ourselves similarly some years ago, when we were dealing with the relatively simple world of microbiological insult, that we would never have a sterile world no matter how we might struggle to obtain it. Even if we obtained it, we might discover that this sterile world was just as hazardous in its stresses and insults as the one which we just left.

One of the positive values of the last two days is in the assembly within a single room of multi-discipline representation. This has not always been easy of accomplishment and has not always been - even when accomplished - as friendly, as interrelated, and even as intercompetitive, as it has been in the last two days.

Complexity of the Environment

A second positive value coming out of this conference is in the illustrations of the complexity of the environment, which naturally follows upon industrialization and urbanization.

In that complexity, one discerns no easy principles or easy axioms. In such simple (or hitherto assumed to be simple) equilibrium as in calcium intake, certainly of interest to a water works man, there are pros and cons, positive and negative values. There are values of fortification in foods as well as disadvantages. The calcium, selenium, molybdenum, sulfate, copper situations all point, not to disaster, but to the fact that the physiological behavior of man is not a simple mechanism which we can evaluate in an easy slide rule effort.

Methods of Analysis and Instrumentation

The third positive value, of course, is the emergence of analytical techniques. This is valuable to record, because as problems in science arise it has almost always been the case that they drive towards the creation of methods of analysis and instrumentation. This is peculiarly true in the field of radioactivity where instrumentation has made a great leap forward in a period of less than a decade. These new directions confront us, and Dr. Gilcreas' observation on the mere size of the new Standard Methods confronts us with an equally difficult problem -- namely, of a superabundance of riches.

I am not saying this facetiously, because in the radioactive field, for example, we are now overwhelmed by radionuclides: their methods of measurement or absence of methods of measurement, their tolerances. I often wonder how a poor water works man of 1961 is going to be able to find his pathway in the morass of the several hundred radionuclides to be considered in raw and finished waters. That these analytical techniques must be developed, no one doubts.

The same suggestion might be made here as a positive part of this conference as was made before a Congressional Committee in relation to radiological techniques: instead of several hundred techniques, we might better ask for one or two or even three gross determinations useful to the practitioner in the sanitary arts. Without it, we are going to find ourselves in great difficulty.

Criteria and Standards

There is a fourth observation which Professor McKee made and which I consider to be a positive part of the conference; that is, the continued insistence on the difference between criteria and standards. Criteria are guides; standards are relatively fixed gospels of autocracy or assumed to come from some remote deity. I sympathize with such differentiation, but it is a losing game.

For example, in the joint report of the Public Health Service and the Corps of Engineers on the Ohio River, we struggled to make certain the distinct differences which Professor McKee pointed out between criteria or guides and standards. After publication, all references to those reports considered the criteria as standards. It is difficult to guard against the use of administrative crutches.

Understanding Biological Effects

A fifth value, of course, is in even the limited amount of information presented on the biological effects of environmental insults and stresses. Those limited amounts are instructive, as to approach, as to laboratory technique, and, more important, as to significance in evaluation. One might be disturbed by them for no other reason than that, from one paper to the other, the pendulum swings from dramatic fear to reassurance. But this, again, is inherent in the present level of understanding of biological effects.

Need for Specificity and Accuracy

May I turn for a moment to the negative features of the conference? These are not intended in any way to depreciate the level of the kind of contribution which this conference, among others, certainly makes. These negative features are important to guarantee that our future operations are within frameworks that aim and ultimately approach a greater degree of specificity and of accuracy.

For example, there is a basic assumption underlying some of the papers that the gross production of additives in a country such as ours is synonymous with a gross increase in danger to people. This assumption still remains to be demonstrated.

Perhaps my best example for those in sanitary engineering is to recall Professor Phelps' text on Public Health Engineering. He has an interesting and instructive chart in the book which shows the increase in telephone poles in the United States since 1890 and the decrease in typhoid fever death rates for the same period. It is an obvious conclusion that the increase in telephone poles reduced the typhoid fever death rate. In a sense, it helped to do so by better communication, by more rapid medical service, by delivery of the ambulance and, in some instances, the hearse. But the direct casual relationship between the telephone poles and typhoid fever is a statistical inaccuracy, which shows up in some of the reasoning during the last two days.

The Need for Basic Data

The second omission, which is noted in some of the papers, is that there are certain positive values in the artificial fortification of food and water, which will probably increase rather than decrease as we understand better the whole metabolic process of man.

A third is the confusion between an association of events and a cause and effect. This is all too common in many of our discussions of the hazards of the environment. It remains to be demonstrated, for example, with insecticides as to how much actually reaches major bodies of water, quantitatively, not qualitatively. And, in turn, of course, what their impact upon the individual may be.

Here, we are confronted with a misfortune of history, and there is nothing we can do about it except to try to catch up as quickly as possible. We lack, as some of the speakers have pointed out, presampling. In other words, what we lack is a base line of environmental status in the various countries in the world for all the elements which we have been discussing; and, of course, all the organic contaminants.

We advocated in the field of radiation the establishment very early of base lines. We were unsuccessful until too late, because the instrumentation for measuring exceedingly low radiation background was not available. By the time it was available, our background situation had changed.

Another negative feature emerges in some of the papers on methodology. Concentration techniques as analytical aids have high value. They should, however, be viewed with a jaundiced eye as demonstrations for propaganda purposes. We have seen a display of materials which have been concentrated one thousand to a millionfold. Unless we have some evidence to indicate exactly what significance such a display has, it runs a little counter to my own scientific instinct. In the absence of the knowledge that we need, these concentrations for methodological purposes are strictly useful. Their application for sales purposes, still remains to be either validated or invalidated. In this very city some 40 to 50 years ago, this same technique was used in attempting to prevent the application of aluminum sulfate in the purification of water, fortunately without success.

A Dearth of Epidemiological Data

A deficiency of even greater import and one which I am sure could not have been corrected within the compass of such a short conference, is the definite dearth of epidemiological evidence throughout the two days. The epidemiological evidence on the biological effects of the things that worry us and should worry us is one of the voids we have to press for correction. And I say "press for" advisedly, because that particular discipline has so far avoided getting entangled in these problems.

There are very good reasons for this avoidance. It is difficult; it is certainly more subtle; it is long term instead of short term. It does not have the specificity of the familar communicable diseases with which they have hitherto dealt. But I still press upon the epidemiologists the necessity of trying to assist in the development of epidemiological tools as well as conclusions. Without them, we are almost permanently restrained in intelligent action.

Directions for the Future

Where do we go from here was one of the questions Dr. Kehoe posed. I am reminded of a recent comment by a prominent statistician at Princton who was asked to do a similar job before the American Statistical Society this past spring. In answer to the confusion of information which had been given to him in the symposium, he said, "Well, some people suggest we ought to go at once to the psychiatrist."

No such pessimistic route is necessary for us. There is a better route, which has been spelled out already by almost every speaker. We need more investigation. This is more than a literary cliche in our particular field. This is an area in which we need expanded development and research of the deepest kind. This is an area in which we need increasing emphasis on our attention to the total ecology of man, with whatever tools we are able to summon up.

We must move toward a rational, conscious equilibrium between the use of technology and its misuse. This relationship has been spelled out before. It is peculiarly a responsibility of industry; a responsibility which I think, in many major industries, is already recognized and practiced, but which must be expanded to all of industry busily engaged in this rapid-fire production of new materials. The production of industrial materials of unknown character, concentration and, certainly, of unknown biological effect is a major joint responsibility of industry. In the next conference of this type, industry should be invited to come and present in greater detail how it intends to cope with this kind of stress over the next twenty years.

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